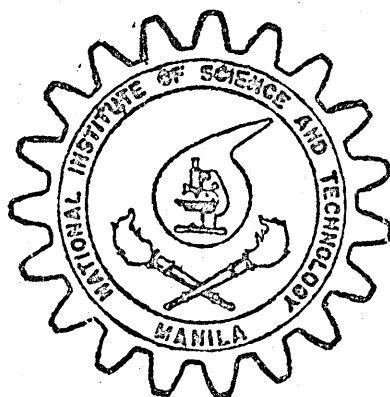


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GERMINATION AND ITS INHIBITION IN COFFEE*

By JOSE R. VELASCO

National Institute of Science and Technology, Manila
and

JORGE GUTIERREZ

Universidad de la Habana, Cuba

ABSTRACT

When fresh and dried coffee fruits and seeds were germinated in sand, the initial germination of fresh seeds without seed coat was obtained about one month after planting; seeds in fresh whole fruits germinated 20 days later. Besides having a late start, the latter germinated more slowly (5 versus 10 per cent per day). Except the seeds without seed coat, the dried material germinated later and slower than their fresh counterparts. Much of the dried berries and seeds rotted.

A germination inhibitor was extracted with 80-per cent methanol from the seed coat of coffee. When partitioned in ethyl acetate-water and chromatographed on filter paper in a system of isopropanol-ammonia-water (10:1:1), its R_f -value was 0.85 to 0.90. It exhibited properties which differed from those established for abscissic acid.

INTRODUCTION

Franco (1966) noted that the literature on the effect of the seed coat on the germination of coffee was contradictory. Mamprim (1947) concluded from field studies that the removal of the seed coat did not necessarily hasten germination. Mes (undated) planted intact seeds as well as seeds with disrupted seed coat.

*The work was undertaken while the senior author was UNESCO specialist in plant physiology at the Universidad de la Habana.



From the results she concluded that coffee had no germination inhibitor. (It was probably meant that the seed coat did not impede germination). Franco (1956-1957) observed that under aseptic conditions, the seeds which were planted with bits of seed coat germinated just as fast as those without seed coat.

This result of Franco appears contrary to that of his earlier study (1946) in which he observed that under aseptic conditions, the seeds with seed coat did not germinate while those without seed coat germinated normally. After 38 days, he removed the seed coat in the former lot and the seeds germinated soon after. Corroborating this finding, Went (1957) concluded from his study that the seed coat delayed germination for at least one week. He was of the opinion that in sand, filter paper or other substrates poor in microorganism, the decomposition of the seed coat is rather slow and the germination may be delayed.

Casual observation in the field may leave ground for the speculation that coffee seeds undergo a latent or dormant period. Thus, the main harvest in Havana, Cuba, occurs in the months of September, October, and November. However, the berries which drop to the ground do not produce massive volunteer seedlings until June and July. Considering that it takes only 3 or 4 weeks for coffee seeds to germinate (Franco, 1966), the delay may suggest that the seed is dormant. Of course, part of the delay could be due to the dry spell from March to May. But there is time as well as sufficient moisture before then, for germination to take place if the seeds were not dormant.

These experiments were conducted to determine the effect of some treatments on the germination of coffee seeds. They were intended to shed light on the presence (or absence) of a germination inhibitor in coffee. As complementary activity, attempts were made to extract and isolate the putative inhibitor and study its properties. The study was conducted in the Jardin Botanico Nacional, Escuela de Ciencias Biologicas, Facultad de Ciencias, Universidad de la Habana, from June to November, 1972.

MATERIALS AND METHODS

1. *Fruits*.—Coffee berries (fruits) were gathered from coffee orchards near the Jardin Botanico Nacional. When not used immediately, they were kept in the refrigerator at near-

freezing temperature. They were a mixture of yellow and red berries of various shapes and sizes.

2. *Test seeds*.—Coffee seeds were used in the study on germination and in a few tests for the germination inhibitor. Other seeds used for the assay of the germination inhibitor were lettuce (*Lactuca sativa*) and Chinese cabbage or bersa (*Brassica pekinensis* Rupr.).

3. *Method of germinating coffee seeds*.—The seeds were laid with the grooved side downward, then covered with 1 to 2 cm of sand. A seed was considered germinated when the "head" consisting of the endosperm and cotyledon, emerged from the sand. The criterion is not precise because the time of emergence is affected by the depth of planting. In later trials a change in criterion was made because some night-foraging insects used to cut the arch of the hypocotyl; hence, the seed was considered germinated when the arch of the hypocotyl surfaced through the sand.

4. *Assay of the inhibitor*.—In a few cases, fresh-coffee seeds without seed coat were used. They were laid in a Petri dish between two sheets of filter paper. Data on germination were taken after 18 days. A seed was considered germinated when the radicle protruded at least 1 millimeter from the outline of the seed.

Most of the assays were made with bersa. The extract was dissolved in 10 ml of water, the pH was adjusted to 6.5 then poured in a Petri dish lined with filter paper. Usually 50 bersa seeds were planted in each dish. At the end of 48 hours, the number which germinated and the length of five hypocotyls were recorded. A seed was considered germinated when the seed coat became ruptured and the hypocotyl protruded out of the seed coat for at least 1 millimeter.

EXPERIMENTS AND RESULTS

1. *Observations on the germination of coffee*.—In the first experiment (July harvest), Lot I consisted of fresh seeds without seed coat, while Lot II consisted of halved berries, i.e., the seed remained enclosed in the seed coat and pulp.

The average time at first germination among the four replicates of Lot I was 36 days (Table 1). It took 44 days for 50

Days after sowing	Replicate							
	Without seed - coat				With seed - coat and pulp			
	1	2	3	4	1	2	3	4
0	A. Number of berries							
	25	25	25	25	25	25	25	25
	B. Number of seeds							
	53	53	57	54	47	51	52	59
	C. Number of filled seed							
	16	27	34	26				
	D. Number of seeds germinated							
34	2							
36-38	3	3	13	6				
41	1	5	2	2				
42	2	4	2	3			1	
43	3	3	3	1				2
44		1	1					
45	1		3	2				3
48-50	3	3	1	3	2	2	8	4
51-52		1					3	2
55					2	7	6	3
56-59					1		4	8
61-62					3	3	2	2
64						2	2	2
66-70		2	1			1	3	4

In comparison, the average time for initial germination in Lot II was 46 days. Since the number of filled seeds in this lot was not determined, the average number of filled seeds in Lot I was arbitrarily used for reckoning 50-per cent germination. Among the three replicates which attained 50-per cent germination, the average time was 58 days.

The number below the broken line indicates seeds germinated but not yet emerged from the sand; the number in parenthesis indicates ungerminated seeds at the end of 70 days; the underlined number marks the half-time (i.e., 50 per cent germinated); the rest rotted.

The second experiment (August harvest) differed from the July harvest in that Lot II consisted of whole fresh berries. Also, a third lot was added (whole dried berries) in order to find if coffee undergoes a secondary dormancy on drying. The occurrence of secondary dormancy was suspected because germination in the field was more than the time it took halved berries, in the first experiment, to germinate.

The dried berries had very erratic germination. One seed germinated in 43 days, another in 46 and a third in 70 days. When they were dug up at 75 days after planting, 37 seeds were found rotten. If the original 25 berries had 48-filled seeds, 8 seeds remained unaccounted for.

Among the seeds without seed coat (Lot I) the average time at initial germination was 31 days after planting. Fifty per cent of the seeds germinated in 36 days, or the rate was 10 per cent per day. Compared with its counterpart in the July harvest, it germinated earlier, its rate and viability were higher, and it had 95 per cent filled seeds as against 47 in the former. (Seeds which sank when immersed in water were presumed to be filled.)

In Lot II the average time at initial germination was 51 days after planting. If each replicate had 48 viable seeds, the average time when 50 per cent of the seeds germinated was 61 days.

An exploratory test which was run in a Petri dish showed that at 18 days after planting all the fresh seeds without seed coat were germinated. Among the dried seeds without seed coat, 60 per cent germinated, 20 per cent were dormant and 20 per cent rotted with fungus growth. The dried seed with seed coat did not germinate.

In another exploratory test comparing seeds without seed coat and those with added fragments of seed coat, all the seeds in the former germinated in 18 days while no germination was noted in the latter. The average hypocotyl length in the former was 5.5 mm; in the latter, 70 per cent had swollen spots at the radicle end, while 30 per cent showed no sign of activity.

In the third experiment, the fresh and dried material consisting of berries, unwashed seeds and washed seeds were compared. It was an attempt to get more data on the effect of drying on germination and to find if the putative inhibitor could be washed off from the seed coat.

The fresh washed seeds attained 50-per cent germination in 48 days, the unwashed seed in 52 days and the berries in 58 days (Table 2). The dried berries and seeds did not attain 50-

TABLE 2.—*Germination of fresh and dried whole berries, unwashed seed and washed seed. (Started September 5, 1972).*

Days after sowing	Fresh material			Dried material		
	Berry	Unwashed seed	Washed seed	Berry	Unwashed seed	Washed seed
A. Number of berries/seeds planted						
	100	209	100	100	215	100
B. Number of seeds germinated						
11-47	3	40	23	18		21
52	26	<u>20</u>	9	18	4	4
53-57	33	20	12	10	4	7
58	<u>12</u>	5				
59-65	61	4	4	8	5	5
66-70	17	2	3	5	1	

	12	2	3	3	2	8
	(6)	(1)		(25)	(3)	(9)

Note: The number below the broken line indicates seeds germinated but not yet emerged from the sand; the number in parenthesis indicates ungerminated seeds at the end of 70 days; the underlined number marks the half-time (i.e., 50 per cent germinated).

per cent germination. When the experiment was terminated at 70 days after planting, the dried berries had 43-per cent germination; the unwashed dried seed, 8 per cent; and the washed dried seeds, 38 per cent.

A fourth trial was started on October 4 with the same treatments as Experiment 3; in addition, the fresh and dried seeds without seed coat were compared. The dried seed without seed coat started to germinate at 29 days after planting, and 50 per cent of the seeds germinated in 34 days. The corresponding data for the fresh material were 33 and 35 days.

2. *Exploratory tests to extract and isolate the inhibitor.*—When lettuce seeds were sown in Petri dish in contact with coffee pulp or the pectinaceous seed coat, the seeds failed to germinate. To pursue it further, the pulp was trituated in the presence of water (1:1 w/v) and the extract was strained through gauze. The seed with seed coat was beaten in water (1:1 w/v) and the extract was likewise strained. The two extracts were assayed for inhibitor using seeds of bersa. Parenthetically, it should be mentioned that bersa is preferable to lettuce because it maintained high viability under our condition of storage, while the latter did not. Furthermore, the germination of lettuce was erratic in the diffuse light of the laboratory.

It may be noted from Table 3 that the undiluted extract from the pulp tended to be more potent than that from the seed

TABLE 3.—*Inhibitory property of extracts from the pulp and the seed coat of coffee. (July 29, 1972.)*

Concentration in mg/ml	Germination ratio	Length of hypocotyl	Other observations
Pulp extract:			
52.9	35/50	0	Just ruptured seed coat; fermenting
18.3	41/50	7 mm	Crooked hypocotyl
8.1	48/50	7 mm	Straight hypocotyl
4.6	49/50	19 mm	Uniformly long hypocotyl
Extract from seed coat:			
36.7	48/50	5	Crooked hypocotyl; fermenting
18.3	49/50	3	Crooked hypocotyl
9.1	50/50	6	Very crooked hypocotyl
4.6	48/50	22	Uniformly long hypocotyl
Control (water)	50/50	29	

coat. This apparent advantage of the pulp disappeared when the concentration of the solids in the extracts were made equal. Being only 40 per cent of the whole seed, the seed coat may be considered a better source of the inhibitor.

Among the other aspects of extraction studied were: (a) the pH of the extractant and the number of extractions, (b) methods of eliminating the pectin from the extract, and (c) methods of separating the inhibitor from the sugar and other constituents. The results may be summarized as follows: (a) Water at pH 3, 6.5, and 9 seemed to have equal capacity to extract the inhibitor. Three changes of water (1:1 w/v) was sufficient to extract most of the inhibitor; (b) Among the methods of separating the pectin — i.e., precipitating with ethyl alcohol, boiling at various pH levels and occluding it in filter paper pulp — the most feasible seemed to be acidifying the extract to pH 3, boiling it for a few minutes, then filtering off the pectin; (c) Attempts to absorb the inhibitor on activated charcoal indicated that this could be effected at pH 6.5 or 9. It was, however, difficult to release it from the charcoal by extraction with water at various pH.

Much time and effort was spent to partition the inhibitor in the aqueous extract at pH 3 with diethyl ether (1:1 w/v) because this is a basic step in isolating abscissic acid from plant extracts. A few of the attempts gave spurious results, but by and large the indications were that the inhibitor could not be partitioned in ether-water. The process was not neat because pectin or some stabilizer in the extract promoted a stable emulsion which took hours to break.

3. *Isolation and characterization of the inhibitor.*—The method of Webb and Wareing (1972) for the extraction and isolation of the inhibitors was tried with some modification. The coffee seed was dried in the sun for 8 to 12 hours. Then the seed coat was separated with a pair of forceps. This was extracted thrice with 80-per cent methanol (1:2 w/v) at 5 to 10°C. for 24 hours, each time. The combined extract was evaporated on a water bath until the residue no longer contained methanol. The aqueous residue was adjusted to pH 8 with 5-per cent sodium bicarbonate and partitioned 4 times with ethyl acetate (1:1 w/v). The combined ethyl acetate fraction was evaporated on a water bath and the residue was picked up with a minimum amount of water. This was bioassayed with bersa

and with coffee seeds. Then an aliquot was loaded on Whatman No. 1 chromatography paper and developed in the solvent system isopropanol-ammonia-water (10:1:1). The solvent was run downwards for 20 cm, then dried at 80°C.

In the assay with bersa, the inhibitor solution allowed 90-per cent germination with 3-mm hypocotyl. In contrast, the control (distilled water) gave 100-per cent germination with 17-mm hypocotyl. In the test with coffee seeds, the lot with the inhibitor did not germinate while the seeds in the control were all germinated at 18 days.

When the chromatogram was bioassayed with bersa, the length in mm of the hypocotyl in the respective 1-cm zones from the origin were: 23, 22, 19, 23, 25, 24, 28, 23, 29, 22, 15, 16, 26, 24, 24, 3, 1, 3, 4, and 13. At 18 cm, three out of four seeds did not germinate. This corresponds to R_f 0.85; the diffuse band had R_f 0.80 to 0.95. When the diffuse band was cut off, re-extracted with methanol, evaporated and the aqueous residue was run again to make a chromatogram, the inhibitor was located largely at R_f 0.85 and a little, at R_f 0.90. According to Webb and Wareing, abscissic acid in this solvent system had an R_f -value of 0.60 to 0.80. Another inhibitor with R_f 0.90 correspond to a neutral compound.

In another trial, sun-dried seed coat (30 grams) was extracted and partitioned accordingly. The residue, after evaporation of ethyl acetate, weighed 138.5 mg. This was dissolved in water and dilution test was made. The concentration giving 80-per cent germination was 15 mg/ml; that giving 20 per cent was 20 mg/ml. The lowest concentration in the test (5 mg/ml) had 10.6-mm hypocotyl compared with 27.0 mm in the control.

DISCUSSION

The indications in this study are that the seed coat delays the onset of germination and elicits a slower rate compared with coffee seeds without seed coat. Franco (1966) stated that in "substrates poor in microorganisms, the decomposition of the seed coat is rather slow and germination may be delayed." However, if the decomposition of the seed coat were a key step in germination, it would be expected that whole berries which harbor profuse microorganisms should germinate earlier than the washed seeds.

Apparently, the capacity of the seed coat to impede some physical process like diffusion or expansion in volume is secondary to its content of some inhibitory substance, because fragments of the seed coat mixed with the seed inhibited germination.

While the dried seeds had erratic and delayed germination, its difference from the fresh seed was not clear enough to confirm the initial supposition that coffee seeds fall into secondary dormancy on drying. It is likely that drying affects some factor which in turn triggers secondary dormancy. For example, the inhibitor in the seed coat might be translocated inwards during the process of drying and might bring about the secondary dormancy of the embryo. Then seeds without seed coat would not become dormant on drying.

In interpreting the results of the bio-assay of the various fractions and preparations of the inhibitor, it may be pertinent to regard the early steps in germination as consisting of two broad categories: (a) the steps leading to the rupture of the seed coat and (b) the steps involved in the elongation of the hypocotyl. An inhibitor which affects the rupture of the seed coat necessarily affects the elongation of the hypocotyl; however, there are substances which act as typical growth inhibitors, affecting only the second category. Most inhibitors prevent the rupture of the seed coat at high concentrations. It may then be useful to establish a parameter for each level of activity. Borrowing the concept of LD_{50} we may conceive of RI_{50} (re-awakening inhibition) and EI_{50} (elongation inhibition). RI_{50} is to mean the concentration of the substance in mg/1 (ppm) which will prevent the rupture of the seed coat in 50 per cent of test seeds, and EI_{50} is the concentration which will inhibit the elongation of the hypocotyl to 50 per cent of that attained by the test seed in distilled water.

Based on the preceding distinction, the RI_{50} of the inhibitor preparation was about 17,000 ppm, and the EI_{50} was less than 5,000 ppm.

Because the inhibitor (1) is stable at 100°C, (2) does not partition into the ether layer at pH 3, and (3) has a Rf-value different from that established for abscissic acid in the solvent system isopropanol-ammonia-water, it seems likely that the inhibitor is not abscissic acid but another substance with related properties.

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STUDIES ON THE PHYSIOLOGY OF PHILIPPINE MINT
(MENTHA CORDIFOLIA OPIZ). II.* EFFECT OF TWO
DIFFERENT LIGHT INTENSITIES ON THE
VEGETATIVE GROWTH AND OIL YIELD

MAGDALENA CANTORIA and MA. VICENTA T. CUEVAS-GACUTAN
University of the Philippines, Quezon City

ABSTRACT

In an effort to induce flowering in *yerba buena* or Philippine mint, the plant was subjected to reduced light intensities. Under the facilities available, the light intensities used were (A) 25 per cent of that outdoors (normal greenhouse light intensity) and (B) 2 per cent of that outdoors (reduced light intensity). The plant did not flower after 35 days of treatment but data on vegetative growth and oil yield were collected. In general, the plant responded more favorably to A than to B. Although there was no significant difference in the oil gland number and oil gland diameter, the oil yield was 28 per cent higher under A than under B on a fresh-weight basis. In a previously reported work, the plant was identified as *Mentha cordifolia* Opiz on the basis of flowering characteristics and composition of the oil.

MATERIALS AND METHODS

Sixteen uniform plants were grown and eight were subjected for 35 days to reduced light intensity by shading the plants with two layers of cheesecloth which were adjusted as the plants grew taller. The rest received normal light intensity inside the greenhouse. The average normal light intensity (A) inside the greenhouse was 25 per cent of that outdoors and the reduced light intensity (B) was 9 per cent of A or 2 per cent of that outdoors.¹ Except for the difference in the intensity of light received, all other conditions were the same for both sets of plants. In addition to the measurements taken in the first work (Cantoria and Mabasa, 1965), leaf length:width ratio, oil gland diameter, and oil yield were determined. Measurement of the oil gland diameter was made with a screw micrometer on the oil glands located in the top margin, base, and middle of the upper and lower epidermis of each leaf indicated.² To study the effect on oil yield, the experiment was repeated using 15 uniform plants per treatment and allowing the plants to grow without removing lateral buds. The oil yield from the

* For previous study, see Cantoria, M., Philip. Jour. Sci. 97: 277-283 (1968).

¹ During the experiment, light intensity was measured with a Toshiba Illuminometer Model 5 on sunny, bright, cloudless days.

² The locations chosen were the same as those chosen for usual pharmacognostical studies of leaf drugs (Cantoria and Mabasa, 1965).

overground parts of 15 plants per treatment was determined by using the National Formulary (American Pharmaceutical Association, 1970) method. The results are shown in Table 1.

RESULTS AND DISCUSSION

Fresh and dry weights were significantly higher and more pairs of leaves were produced under ordinary greenhouse light (A) than under reduced light (B). A similar dry weight increase due to an increase in light intensity has been reported for the woodland annual, *Impatiens parviflora* by Evans and Hughes (1961) and for young apple trees by Maggs (1960). This increased dry weight as a result of higher intensity of light is explained by Warburg and Burk (1950) to be a consequence of an increased rate of photosynthesis. Newton (1963), however, reports that in cucumber, dry weights are influenced most by the amount of radiation received and that the dry-weight increase under an interaction of light intensity and day length is influenced much more by the size of the leaf surface than by the expected increased rate of photosynthesis. Bean (1964) found that in *Dactylis glomerata*, decreasing the light intensity decreased the growth rate and the rate of tiller production.

Dry weights of individual leaves and of individual internodes were significantly higher under A than under B. Similar increases in the dry weight of the leaves of Marquis wheat have been obtained by Friend *et al* (1962) and in ryegrass by Mitchell (1953) as a result of increased light intensity. In apple plants, Maggs (1960) reported that the greatest weight of individual leaves was obtained under full sunlight. Coombe (1967), working on *Impatiens parviflora*, reported that there is a marked tendency for shaded plants to have a slightly higher proportion of their dry weights in leaves compared to those grown in the clearing. Forsyth and Hall (1965) observed no difference in internode dry weight of the lowbush blueberry, *Vaccinium angustifolium*, under the three light intensities they used.

Maximum growth was achieved earlier under reduced light intensity (B) than under A but was lower as based on dry weight production. There was a decrease in dry weight of leaves and internodes above the 11th leaf under B, while under A, the dry weights continued to increase. This increased growth

was reflected in the increased dry weights of the internodes upwards under A while there was a marked decrease under B above the 11th internode.

Although, generally, there was no significant difference in the oil gland number and oil gland diameter, the results were not consistent. There was, however, a consistently higher oil gland number but smaller oil gland diameter in the lower epidermis than in the upper epidermis.

The oil yield of 15 plants grown under A was 28 per cent higher than that under B, on a fresh-weight basis. These findings are consistent with the generalization by Flück (1963) that plants produce their maximal content of active principles under those climatic conditions which allow the optimum development of the plant itself.

Leaf length and leaf width were significantly greater under A. In the 14th leaf, a difference in shape was noted between the two treatments, the leaf being elongated (higher length:width ratio) under A and slightly rounded (lower length:width ratio) under B. It seems that only after prolonged exposure to lower light intensity does leaf shape change. According to Bensink (1960), the length:width ratio in lettuce leaves increases under low light intensity and this is due to midrib elongation and reduction in leaf blade development. Friend *et al* (1962, 1965) found that in Marquis wheat, an increase in light intensity is accompanied by increased leaf width and decreased leaf length. They explain these effects as being caused by a change in cell dimensions rather than by changes in the number of cells. Mitchell and Soper (1958) also noted in *Lolium perenne* and *Paspalum dilatatum* that an increase in light intensity shortens and widens leaves. In *Lolium* spp., Mitchell (1953) observed that the length and median width of the leaves follow a complex pattern in response to light and temperature and acted as independent variables. Conditions which influence leaf length may or may not have an influence on leaf width. The median width of successive leaves decreases as light intensity is reduced while leaf length of each succeeding leaf increases with increases in temperature. In young apple trees, Maggs (1960) found that heavy shade produced the largest leaves. Murray and Nichols (1967) also noted in cocoa that shading increased leaf area both of individual leaves and of total leaf area per plant. Early in the growth of barley, leaf area was depressed by shad-

ing which slowed leaf production but did not induce smaller leaves, according to Kamel (1958). Bean (1964) noted that light intensity affected the area of leaves of *Dactylis glomerata* but had no effect on the number of leaves per tiller.

The effect of B is first evidenced in the etiolation of internodes and somewhat later, in the decreased growth of leaves. This can be seen by comparing lengths and dry weights of internodes and the lengths, widths, and dry weights of leaves under the two treatments. Under B, etiolation could be detected starting with the 5th internode while in the 5th leaf no significant differences were found. Significant differences were noted starting with the 8th leaf, growth being depressed under B. Lockhart (1961) reports that the growth of the axis of higher plants is high at a light intensity of approximately 40,000 lux but is reduced at a higher or lower intensity. Grime (1967) noted that increase in height as a response to shading is due to the presence of extension sites, the number, distribution, sensitivity, plasticity, and stability of which vary in different species. Plants with limited height in the shade have their extension sites available for only a short period of time. In apples, Maggs (1960) observed that shading reduces internode length and consequently, the length of new stems. He correlated slower growth at low light intensity with deficiency of photosynthetic products, and growth inhibition at high intensity with gibberellin deficiency. The increase in length of the 14th internode under A is not a case of etiolation but is due to increased growth since it is accompanied by an increase in dry weight and also by an increase in size of the 14th leaf. Hughes (1967), working on *Impatiens parviflora*, found out that shading increases specific leaf area but reduces leaf:weight ratio. Loach (1967) further noted that in all the species he used, chlorophyll content per unit leaf weight was least in open-grown leaves and increased with successive degrees of shade. Shade leaves were also observed to contain less tissue per unit leaf area than sun leaves. According to Kamel (1958), as the barley plant grows, the photosynthetic apparatus becomes more efficient, thus bringing about increased growth. In *Dactylis glomerata*, Bean (1964) observed varied responses to light intensity at different growth stages. Since the growth of the green tissue at each light intensity varied little over a wide range of leaf area index, it seems that rye grass swards may be able to increase the ef-

TABLE 1.—Growth of *Mentha cordifolia* Opiz under two different light intensities.

		A	B	$\frac{A-B}{B} \times 100\%$
		Normal Intensity	Reduced Intensity	
Total	Fresh weight (g)	18.90 $\pm$ 1.17	10.28 $\pm$ 0.06	84
	Dry weight (g)	2.10 $\pm$ 0.07	1.06 $\pm$ 0.07	98
Number of pairs of leaves		24.80 $\pm$ 0.02	22.80 $\pm$ 0.25	9
Percentage oil yield of fresh weight		0.147	0.117	--
Oil gland number ^b	Upper epidermis	3.98 $\pm$ 0.08	4.59 $\pm$ 0.18	--
	5th Lower epidermis	9.08 $\pm$ 0.24	9.44 $\pm$ 0.23	--
	Upper epidermis	4.08 0.13	5.29 0.18	--
	8th Lower epidermis	10.08 0.28	10.15 0.21	--
	Upper epidermis	5.34 0.16	5.10 0.38	--
	11th Lower epidermis	11.20 0.18	10.37 0.19	8
	Upper epidermis	4.08 0.13	5.25 0.19	27
	14th Lower epidermis	10.68 0.30	11.32 0.31	--
	Upper epidermis	0.132 0.0014	0.134 0.0013	--
	5th Lower epidermis	0.130 0.0009	0.133 0.0008	--
	Upper epidermis	0.149 0.0008	0.145 0.0012	--
	8th Lower epidermis	0.147 0.0010	0.142 0.0014	4
Oil gland diameter (mm) ^c	Upper epidermis	0.147 0.0010	0.146 0.0016	--
	11th Lower epidermis	0.145 0.0013	0.144 0.0017	--
	Upper epidermis	0.145 0.0014	0.141 0.0014	--
	14th Lower epidermis	0.143 0.0010	0.136 0.0004	4
Leaf	5th Dry weight (mg)	19.30 1.56	17.10 1.04	--
	Length (cm)	4.05 0.15	4.16 0.11	--
	Width (cm)	3.28 0.13	3.14 0.09	--
	Length: width	1.25 0.05	1.32 0.02	--
	8th Dry weight (mg)	40.60 2.02	25.00 0.98	58
	Length (cm)	6.10 0.17	5.28 0.12	16
	Width (cm)	4.41 0.08	3.95 0.08	12
	Length: width	1.39 0.02	1.34 0.03	--
	11th Dry weight (mg)	48.80 1.43	26.90 1.69	81
	Length (cm)	5.45 0.18	7.02 0.11	28
	Width (cm)	4.95 0.03	4.22 0.09	16
	Length: width	1.40 0.02	1.32 0.04	--
	14th Dry weight (mg)	54.10 2.39	25.40 1.38	113
	Length (cm)	7.39 0.16	5.30 0.18	39
	Width (cm)	5.08 3.98	3.98 0.09	28
	Length: width	1.46 0.02	1.35 0.02	8
Internode	5th Dry weight (mg)	26.60 $\pm$ 0.84	21.20 $\pm$ 1.27	21
	Length (cm)	1.65 $\pm$ 0.12	3.22 $\pm$ 0.15	95
	8th Dry weight (mg)	44.70 $\pm$ 3.15	30.40 $\pm$ 1.69	47
	Length (cm)	3.11 $\pm$ 0.02	4.05 $\pm$ 0.12	30
	11th Dry weight (mg)	68.10 $\pm$ 3.61	37.70 $\pm$ 2.16	81
	Length (cm)	4.89 $\pm$ 0.21	5.22 $\pm$ 0.28	--
	14th Dry weight (mg)	70.48 $\pm$ 1.58	32.00 $\pm$ 1.27	120
	Length (cm)	5.34 0.09	4.89 0.13	11
Palisade number	6th leaf	18.47 0.75	19.65 0.84	--
	13th leaf	22.70 0.44	23.76 0.60	--

Mean of 8 plants with the standard errors of mean (1).

^aValues are presented where differences between A and B are significant at 1 per cent level.

^bPer 0.48 mm².

^cThe figures represent the means of 96-128 measurements, with the standard error of the mean.

iciency with which they use light by adaptation to a changing light environment as growth proceeds.

The Philippine mint may be considered a shade-tolerant species as it was observed to grow well inside the greenhouse, the normal light intensity of which is 25 per cent of that outdoors. Loach (1967) explained that these shade-tolerant species could photosynthesize efficiently under low light intensities and correspondingly reduce their rate of respiration. This also explains the ability of such plants to survive conditions of low light intensities. Coombe (1967) also mentioned that the success of the shade plant *Impatiens parviflora* depends mostly on its remarkable plasticity of leaf area per unit leaf weight as well as its positive dry-weight gain at very low light intensity.

Many tropical plants were observed by Murray and Nichols (1967) to have light intensity as a growth factor. They further noted that the light levels at which certain tropical plants give their maximum yield is a function of their mineral status. If the mineral nutrition is poor, shade is necessary. Shading, they suggested, slows down the metabolism of the leaf and reduces any imbalance between photosynthetic products and their mineral supply.

The plants did not develop flowers after 35 days under the two light intensities used in this experiment.

SUMMARY AND CONCLUSIONS

Growth of Philippine mint (*Mentha cordifolia* Opiz) under two different light intensities in the greenhouse was studied. On the basis of dry-weight production, growth was better under normal greenhouse light intensity (25 per cent of that outdoors) than under the reduced light intensity (2 per cent of that outdoors). There was no distinct effect on the number of oil glands per unit area nor on the size of the oil glands. Prolonged exposure to reduced light intensity resulted in a change in leaf shape and in etiolation. After 35 days of treatment, the plants did not develop flowers under the two light intensities used.

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A NOTE ON THE CONTROL OF POSTHARVEST DISEASES OF FRUITS WITH BENOMYL AND THIABENDAZOLE*

By N. D. BONDAD¹
University of the Philippines, College, Laguna

ABSTRACT

Benomyl provided a better control of decay in nondegreened citrus ('Szinkom') fruits than thiabendazole. *Phomopsis citri* was found to be the major causal organism. Thiabendazole at 200 ppm did not satisfactorily control diplodia stem-end rot of mango ('Piko'). Further development of cushion rot in heavily infected bananas ('Lakatan') was checked by 500-ppm thiabendazole in green but not in ripe fruits. Results with tomatoes (unknown cv.) are not conclusive due to uncertainty of previous handling conditions.

INTRODUCTION

In the Philippines, the hot and humid climate causes food to perish very quickly. Various estimates (Acena, 1967; Cadiz, 1969; Pantastico *et al*, 1970) indicate that about 30 to 50 per cent of harvested fruits and vegetables are lost annually. Control measures have never been fully considered as shown by the meager information on the control of postharvest diseases of vegetables (Bondad and Pantastico, 1970). Practically no information is available on fruits.

Chemical treatments aimed at reducing spoilage should be considered as supplements to refrigeration (Smith, 1962). However, in the Philippines where refrigeration is not a common practice, chemical treatments may be considered as primary measures in the storage of perishables. Control of spoilage using two newly introduced fungicides, benomyl and thiabendazole, has been the subject of numerous investigations in recent years. There is an increasing volume of literature relating to effectiveness of the fungicides in controlling certain postharvest diseases (Du Pont, 1969; Eckert, 1969; Merck, 1970; Meredith, 1971) but no work under local conditions has been published. Results of preliminary experiments are presented in this paper.

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¹ Present Address: Philippine Council for Agricultural Research, Los Baños, Laguna.

MATERIALS AND METHODS

General.—The water-insoluble fungicides were applied as suspensions on naturally infected fruits with continuous agitation. Unless otherwise specified, the dipping time was 2 minutes. In all cases, the fungicides were dried on fruits without subsequent rinsing. To prevent dehydration, polyethylene bags or sheets were used to store or ripen the fruits. Except for bananas, treatments were done at the day of harvest or procurement.

Citrus ('Szinkom').—Mature green fruits from the Agromy orchard were dipped in 500-ppm benomyl (courtesy of Du Pont as *benlate*) or thiabendazole (courtesy of Merck Sharp and Dohme as *Tecto 90*) with 0.1-per cent *bacta-kleen* (surfactant supplied by Merck). Another set was degreened first with 0.3-g calcium carbide/liter for 24 hours then dipped in the fungicides. Fruits were packed in perforated (2 holes, 4 mm dia.) polyethylene bags (1.5 mils, 9 x 12 in.) then stored at 79 to 83°F.

Mango ('Piko').—Fruits picked from a commercial plantation were dipped in 500-ppm thiabendazole with 0.1-per cent *triton X-100* (surfactant from Rohm and Haas). After air drying, fruits were placed in 3.5 liter capacity jars covered with 1.5 mil polyethylene sheets then stored at a laboratory temperature of 72 to 76°F.

Banana ('Lakatan').²—The fruits were air-freighted from Davao to Manila under commercial packing conditions. All fruits were infected with cushion rot on arrival in College, Laguna 4 days after harvest. Hands of green and ripe fruits were divided into equal parts. One half was dipped in 500-ppm thiabendazole with 0.1-per cent *bacta-kleen* and the other half in tap water with the same amount of *bacta-kleen*. Treated and control fruits were packed in perforated (4 holes, 4 mm diam.) polyethylene bags (1.5 mils, 10 x 18 in.) then stored at 72 to 76°F.

Tomato (*unknown cv.*).—Ripe fruits from a local market were dipped for 5 minutes in 100- to 500-ppm thiabendazole with 0.1-per cent *triton X-100*. After air drying, fruits were packed in perforated (8 holes, 4 mm diam.) polyethylene bags (1.5 mils, 9 x 12 in.) then stored at 85 to 95°F.

² A Philippine cv. different from 'Lakatan' of Central America (Valmayor, 1971).

RESULTS AND DISCUSSION

Citrus.—Table 1 shows that benomyl and thiabendazole were equally effective in controlling the development of decay in nondegreened fruits during the first 13 days. However, benomyl provided a better control of decay than thiabendazole during the latter days of infection. McCornack and Brown (1969) also found that *benlate* (benomyl) was superior to *Mer-tect* 260 (thiabendazole) in decay control of 'Hamlin' oranges but with other types of citrus fruits, benomyl was not consistently better than thiabendazole (Harding, 1968). Thiabendazole

TABLE 1.—Development of decay in Szinkom citrus fruits dipped in 500-ppm benomyl or thiabendazole (dried on fruits) for 2 minutes then stored in perforated polyethylene bags at 79 to 83°F.

Days after treatment	Decayed fruits* (Per cent)					
	Control		Benomyl		Thiabendazole	
	non-degreened	degreened	non-degreened	degreened	non-degreened	degreened
9	0	2.2	0	1.9	0	0
13	14.5	17.8	0	19.7	0	19.7
19	34.0	29.8	1**	32.5	7.5**	39.7
29	92.5	77.0	9**	58.5**	43.0**	95.0

* Means of five replications of 25 fruits each.

** Significant at 0.05.

had no effect on control of decay in degreened fruits. In most cases, the condition of untreated fruits was better than those dipped in thiabendazole. Benomyl was effective in controlling decay of degreened fruits only during the 29th day. These might probably be due to a rapid multiplication of decay microorganisms resulting from a delay of treatment and rise in temperature during degreening. Microscopic examination of infected fruits by Dr. Lina Ilag showed that *Phomopsis citri* was the major fungal pathogen in both degreened and nondegreened fruits. With naturally infected 'Valencia' oranges, *Phomopsis citri* was also found to be the predominant fungus controlled by *benlate* under Florida conditions (Smoot and Melvin, 1969).

Mango.—Thiabendazole at 200 ppm did not satisfactorily control diplodia stem-end rot (identified by Mr. P. M. Halos). Ten days after treatment, control and treated fruits had 25 and 23-per cent surface decayed, respectively. Stem-end rot started as small brown to black areas at the base of the peduncle progressively infecting the fruit toward the blossom end. Even the interior of unaffected surfaces was discolored. Similar symptoms of diplodia rot were described by Alicbusan and Shafer (1958), and Halos and Divinagracia (1970).

TABLE 2.—Effect of 500-ppm thiabendazole on the development of decay in lakatan bananas air-freighted from Davao to Manila then land-transported to College, Laguna.*

Treatment	Infected fruits**	After days
	Per cent	
	5	8
Green		
control	6.0	54.1
500-ppm thiabendazole	1.0	2.0
Ripe		
control	38.0	100.0
500-ppm thiabendazole	20.0	100.0

* The time delay between harvest and treatment was 4 days.

** Infection of cushion rot that developed from the crown to about 50 per cent of individual fingers. Averages of 5-replicated hands of 6 to 10 fruits each.

Banana.—A satisfactory control of decay in 'Cavendish' banana was obtained by Long (1970a) even after a delay of 36 hours between harvest and treatment with 500-ppm thiabendazole. With a delay of 4 days, the same concentration of thiabendazole was very effective in preventing further development of cushion rot (identified by Prof. A. N. Pordesimo) in heavily infected green fruits (Table 2). Eight days after treatment, control and treated fruits lost about 87.9 and 3.9 per cent marketable weights, respectively. A lower concentration of

100-ppm thiabendazole was also effective in controlling cushion rot in 'Robusta' bananas (Phillips, 1970).

Thiabendazole was not effective in controlling decay of ripe fruits. Both control and treated fruits were nonmarketable 8 days after treatment. These support the contention that ripe fruits are more susceptible to fungal infection than green fruits (Biale, 1954). Successful control of postharvest diseases of bananas with thiabendazole has also been carried out by other workers (Bailey *et al*, 1970; Burden, 1969; Long, 1970a, 1970b; Phillips, 1970; Scott and Roberts, 1967; Shillingford, 1970) using green fruits.

Latent infection of anthracnose was apparently not controlled by thiabendazole as this was later (after 8 days) observed to be severe in both treated and control fruits. However, *in vitro* tests (Long, 1970a; Scott and Roberts, 1967; Shillingford, 1970) show that thiabendazole can control the sporulation of *Gloeosporium musarum*, a causal fungus of anthracnose (Meredit, 1969).

Tomato.—Complete control of tomato spoilage was attained when ripe fruits were dipped in 100-ppm thiabendazole. While 40 per cent of control fruits were already decayed, all fruits treated with thiabendazole were still in good marketable condition 7 days after treatment. Weight loss due to spoilage, transpiration and respiration was 6.2 g/kg for treated and

TABLE 3.—Loss in weight of tomato (unknown cv.) treated with varying levels of thiabendazole.

Thiabendazole (ppm)	Weight loss (g/kg)*			After days
	7	9	12	
100	6	10	16	
200	437	563	700	
400	670	778	788	
500	723	775	776	

* Due to respiration, transpiration and removal of infected fruits. Averages of four replications of 20 fruits each

643.1 g/kg for control fruits. As soon as treated and control fruits were removed from the polyethylene bags, no further development of decay was observed showing that the high relative humidities inside the bags contributed to the growth of spoilage microorganisms. Fifteen days after treatment, all fruits were nonmarketable due to shrivelling. Higher concentrations of thiabendazole (200 to 500 ppm) appeared to be injurious (Table 3). However, this and the previous observations on tomatoes are not conclusive since the source and previous handling conditions of the fruits were not known.

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A STUDY OF THE PROCESS OF PRODUCING COPPER SULFATE AND COPPER SALTS FROM CHALCOPYRITE

By PILARITA A. COCJIN and FILIPINA S. DE DELA FUENTE
National Institute of Science and Technology, Manila

FOUR TEXT FIGURES

ABSTRACT

A series of experiments was conducted to determine the optimum conditions under which copper sulfate of industrial quality may be obtained from chalcopryrite. The data gathered disclosed that the CuSO_4 could be extracted from chalcopryrite by first roasting the ground ore at 400°C for 6 hours. The roasted ore is then leached with tap water and subsequently filtered. The filtrate which contains CuSO_4 and $\text{Fe}(\text{OH})_3$ is evaporated to half its volume to separate the two salts. The $\text{Fe}(\text{OH})_3$ residue is removed by filtration. Finally, the CuSO_4 solution is concentrated until the specific gravity of the solution is between 1.2 to 1.4. At this point CuSO_4 starts to crystallize out.

INTRODUCTION

The Philippines is rich in mineral deposits which find their way into the foreign market either in their crude form or beneficiated to a certain level of purity. In the case of copper ores, we have an abundant deposit of all types in all parts of the archipelago. The vein and vein form replacement deposits consisting of chalcopryrite (CuFES_2), bornite ($2 \text{ Cu}_2\text{S}$ and Fe_2S_3) and quartz are predominantly found in Mt. Province, Zambales, Masbate, Batangas, Panay, Pangasinan, Marinduque, Ilocos Norte, and Cebu. Copper mineralization is being undertaken by the following mining companies: Lepanto copper mine of Mt. Province; Mindanao Lode Mines, Inc. of Cabagan, Zambales; Botolan Copper Mine of Botolan, Zambales; Baleno Copper Mines Co. of Masbate; Lobo Copper Mine of Batangas; Pilar copper deposits in Panay; Polymines of Pangasinan; Buangan copper deposits of Marinduque; Philippine Copper Mines, Inc. of Ilocos Norte; Copper-zinc deposits of Kanapnapan, Toledo, Cebu, and Manila Mining Corporation of Masbate.

The disseminated and stock work deposits consist of chrysocolla ($\text{CuO} \cdot \text{SiO}_2 \cdot 2\text{H}_2\text{O}$), malachite ($\text{CaCO}_3 \cdot \text{Cu}(\text{OH})_2$), azurite ($2 \text{ CuCO}_3 \cdot \text{Cu}(\text{OH})_2$), tetrahedrite ($\text{Cu}_3\text{SbS}_3 \cdot x(\text{Fe}, \text{Zn})_6 \text{Sb}_2\text{S}_6$) and chalcopryrite. The biggest mining companies at present mining this type of copper deposits are Atlas Consolidated Mining and Development Corporation of Cebu, Dilalongan copper

deposit in Quezon, and Cook and Cambindol copper deposits of Marinduque. We also have abundant deposits of this type of copper ore in Sipalay, Negros Occidental; Sto. Tomas, Mt. Province, and in Bacot, Toledo, Cebu.

The cupriferous pyrite deposits consist of chalcopyrite chalcocite (Cu_2S), bornite and tetrahedrite. The Hixbar Mines of Rapu Rapu Island, Cebu; Philippine Iron Mines of Camarines Norte; the Barlo Deposit in Pangasinan; and the Tolosa Mining Corporation of Albay are so far the biggest mining companies mining this type of copper deposits. Marinduque has six mining companies involved in mining cupriferous pyrite deposits; namely, Mineral Resources Company, Bagacoy, Pauli, Isaw, Sabong, and Biminga.

On the other hand, chemicals such as copper sulfate, cuprous chloride, copper acetate, cupric aceto arsenite or Paris green and other agricultural copper salts needed by our local industries, researchers, agriculturists and technical schools are still being imported.

TABLE 1.—*Volume and value of imported copper sulfate, 1960-1967 (In-thousand pesos, FOB).*

Year	Tons	Pesos (FOB)
1960	188.4	106
1961	80.4	43
1962	131.5	104
1963	247.4	192
1964	17.4	32
1965	12.1	13
1966	-	-
1967	20.2	28

Source: Central Bank of the Philippines

Copper sulfate leads as an industrial copper compound. It has extensive agricultural application as a fungicide (Table 2) and as soil additive designed to prevent copper deficiencies in crops or animals or compound crop yields. It is used as a mordant in dyeing, in electric batteries, in process engraving and lithography, as a copper plating bath, and in preparing a number of compounds used for the destruction of snails and slugs

TABLE 2.—Volume and value of imported fungicides and agricultural insecticides and formulations, 1963-1968 (In-thousand pesos, FOB).

Year	Fungicides		Agricultural insecticides and formulations	
	Tons	Pesos (FOB)	Tons	Pesos (FOB)
1963	171.3	704.0	1,189.3	2,235.2
1964	175.0	632.4	1,088.2	2,133.6
1965	152.5	556.8	1,204.9	2,892.0
1966	148.0	540.0	1,261.5	3,177.6
1967	146.7	788.4	10,712.8	10,320.0
1968	487.0	727.6	1,983.4	6,146.4

Source: Central Bank of the Philippines

and low form of insect pests. A preparation used to combat plant diseases is a 2-per cent solution in lime water. Cupric aceto arsenite or Paris green (Table 3) and cupric arsenite or Schule's green both derived from copper sulfate are used as wood preservatives and as larvacides for mosquito control. They are also used as pigments and insecticides.

TABLE 3.—Volume and value of imported "Paris Green" copper aceto arsenite, 1963-1968 (In-thousand pesos, FOB).

Year	Tons	Pesos (FOB)
1963	376.8	490.8
1964	379.4	807.6
1965	352.6	564.4
1966	122.6	269.6
1967	405.9	680.8
1968	376.8	490.8

Source: Central Bank of the Philippines

With the direct uses of copper sulfate plus its use as a raw material for other chemicals, and with the premise that Paris green is manufactured locally and considering that about 1 ton of hydrated copper sulfate is needed to manufacture 1

ton of Paris green, a conservative estimate of the apparent demand for copper sulfate is in the range of 250 to 300 tons per year. Current quotation of copper sulfate given to the NIST by a local dealer is ₱10.75 per kilo.

This project, therefore, was undertaken to study the feasibility of processing copper sulfate and other copper salts from chalcopyrite. This will not only help in the exploitation of the country's natural resources but it will also serve as a dollar-saving industry.

REVIEW OF LITERATURE

Several processes of producing copper salts from the ore were considered. Leutie and Fricon (1943) suggest the preparation of copper sulfate from any copper ore by first converting the ore into its soluble form by digestion with aqua regia, then separating copper from the acidic solution by electrolysis, oxidizing the copper to cuprous oxide or acidification with concentrated sulfuric acid to produce copper sulfate. Electrolysis, however, has already been proven to be very expensive for commercial scale production.

Gorter and Dever's (1962) method is, according to the authors themselves, not only impractical but also uneconomical because it suggests the use of the very expensive tuton salt for separating copper from the ore. Besides, tuton salt is not available locally.

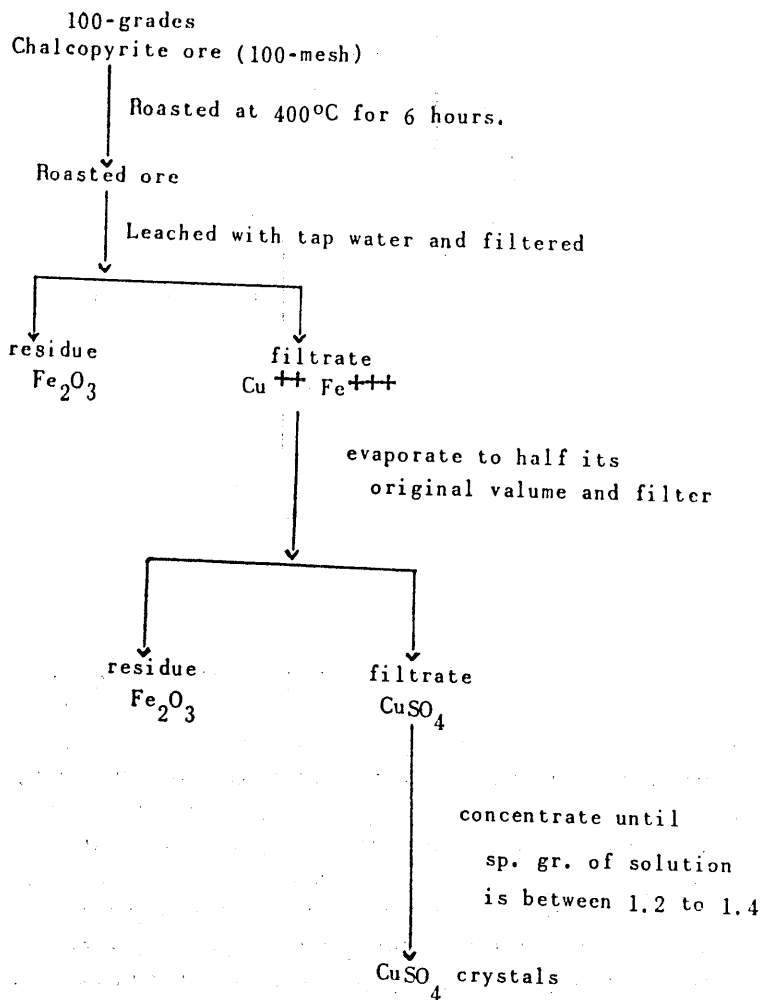
Therold Moeller describes the conversion of the copper from the ore into complex ion prior to extraction. One problem arises, however. Once the copper complex ion is formed, it is very difficult to convert it to copper sulfate and other copper salts (Moeller, 1944). Besides, some of the copper may be lost during the conversion.

The process which is applicable to chalcopyrite involves the roasting of the ore, thus converting the copper sulfide to copper sulfate, then separating the copper from the iron oxide present in the ore by leaching and filtration and finally concentrating the aqueous extract to crystallize out the copper sulfate. This is a general process for cupriferous pyrite deposits because there is no need to introduce sulfur to the ore. If this process, however, will turn out to be uneconomical then modification will later be made.

then analyzed to determine the per cent purity. The per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was determined iodometrically.

The above procedure was later modified until an economical procedure was formulated. Method 2 was conceived to attain such objectives. No chemicals or reagents were needed for the extraction. The optimum conditions were obtained after undertaking several experiments.

Method 2 - no chemicals needed:



Using Method 1, the following experiments were undertaken:

Experiment No. 1. Effect of Roasting

Sample No.	Yield $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Recovery
	Per cent	Per cent
1 with roasting	58.6	42.09
2 without roasting	10.8	7.63

Experiment No. 2. Effect of using different mineral acids for leaching.

Sample No.	Acid used for leaching	Yield $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Recovery
		Per cent	Per cent
1	Hydrochloric acid (HCl)	58.6	42.09
2	Nitric acid (HNO_3)	42.3	30.31

Experiment No. 3. Effect of using different alkali for the separation of copper from iron.

Sample No.	Alkali used	Yield $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Recovery
		Per cent	Per cent
1	NH_4OH	58.6	41.28
2*	NaOH	28.6	18.21

*The residue collected after precipitating the iron with NaOH which was supposed to be purely $\text{Fe}(\text{OH})_3$ after adding enough NaOH had a light blue color indicating therefore that some copper still remained in the residue.

Experiment No. 1 of Method 1 shows that roasting is a very important step in the extraction and, therefore, the optimum roasting temperature was determined. From the accompanying data as well as the graph, it is shown that the best roasting temperature for chalcopyrite is $400 \pm 5^\circ\text{C}$. This data was obtained using Method 2.

Experiment No. 1. Effect of roasting temperature.

Sample No.	Temperature of roasting $^\circ\text{C}$	Yield Per cent	Recovery Per cent
1	850	8.9	6.34
2	650	31.0	22.17
3	550	38.6	27.58
4	450	43.3	30.97
5	400	53.5	38.26
6	350	40.8	29.18

Using the optimum roasting temperature, the best roasting time was determined. From the accompanying table, and graph, it can be seen that the longer the time of roasting the

Experiment No. 2. Effect of varying the time of roasting temperature — $400 \pm 5^\circ\text{C}$.

Sample No.	Time of roasting hrs	Yield Per cent	Recovery Per cent
7	2	10.5	7.51
8	3	53.5	32.26
9	4	59.1	41.55
10	5	59.6	42.62
11	6	59.8	42.77
12	7	60.0	42.91
13	8	60.8	43.48

higher is the per cent recovery. However, after 5 hours of roasting the amount of recovery is not much compared to the amount of electricity consumed in roasting. Therefore, the optimum time of roasting was taken as 5 hours.

The crystals produced in the first two sets of experiments were relatively impure because the method of crystallization did not follow a definite pattern. A third set of experiments was therefore conceived, the objective of which was to determine the specific gravity at which the highest purity of the crystals could be obtained.

Experiment No. 3. Effect of specific gravity of the concentrated solution on the amount and quality of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystals formed.

Time of roasting — 6 hours

Temperature of roasting — $400 \pm 5^\circ\text{C}$.

Per cent purity of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ obtained — 99.5 per cent.

Sample No.	Specific gravity of concentrated solution	Yield	Recovery
		Per cent	Per cent
14	1.090	no crystals	
15	1.166	no crystals	
16	1.203	47.87	31.40
17	1.210	49.95	32.23
18	1.250	51.53	36.54
19	1.340	72.42	51.79
20	1.400	89.91	64.30

Since $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystallizes between the range of the specific gravity from 1.20 to 1.40, a study was made noting the effect of specific gravity on the amount and quality of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystals formed. As shown in the above table, the higher the specific gravity, the higher is the percentage recovery.

It might be noted from the above results that roasting for over 2 hours at low temperature is detrimental to the process of extraction because the sulfur content of the ore is burned excessively at this condition.

Experiment No. 4. Effect of longer roasting at low temperature on the per cent yield of copper sulfate.

Sample No.	No. of hours of roasting at			Per cent yield
	100°C	200°C	300°C	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
1	4	1	1	30
2	2	1	1	28
3	3	1	1	20
4	1	2	1	38
5	1	3	1	21
6	1	1	2	20
7	1	1	3	19.6

In order to produce high quality crystals, another study was made wherein crystals were collected at various stages of crystallization. The best quality crystals were found to grow on the first day of crystallization as shown in the above table. This experiment also explains the result obtained in Experiment No. 3, that the higher the specific gravity, the faster is the rate of crystallization and, therefore, the higher is the percentage purity of the copper sulfate crystals formed whereas the

Experiment No. 5. Effect of slow crystallization on the purity of the $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystal formed.

Sample No.	Day crystals were collected	Ave. yield
		<i>Per cent</i>
1	1st	90.02
2	2nd	82.14
3	3rd	80.00
4	4th	68.97
5	5th	40.06

lower the specific gravity, the lower is the rate of crystallization and sometimes impurities crystallize together with the $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ producing poor quality crystals.

Another modified standard method was tried wherein different concentrations of HCl were used for leaching. The result is shown in the accompanying table and graph.

Method 3 — Leaching with different concentrations of HCl. Effect of using different concentrations of HCl as leaching agent.

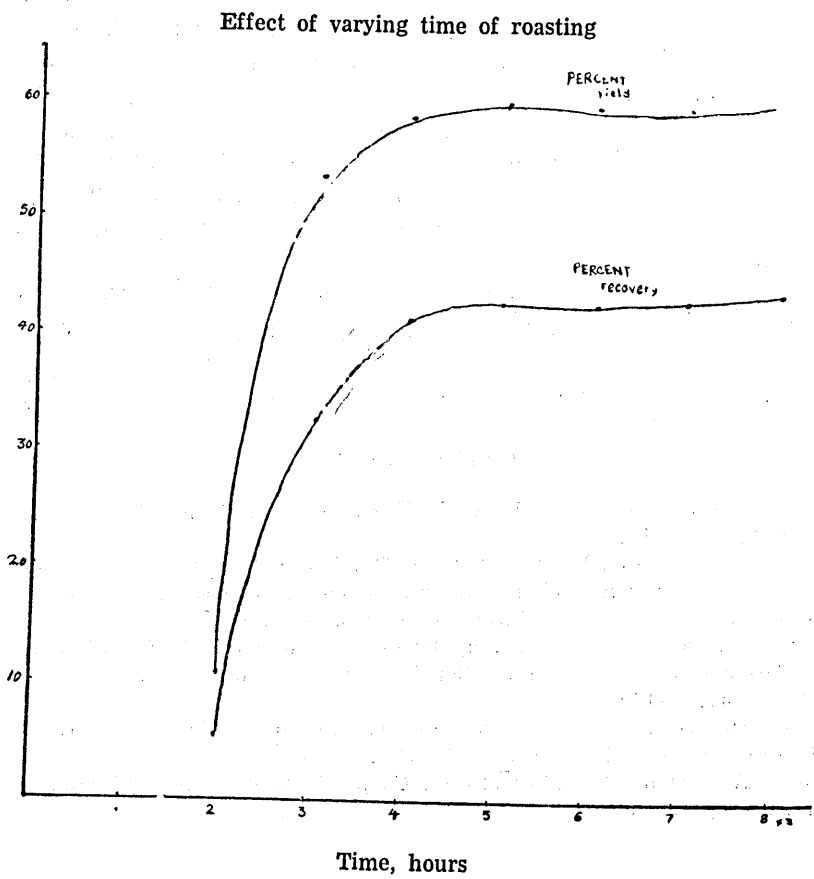
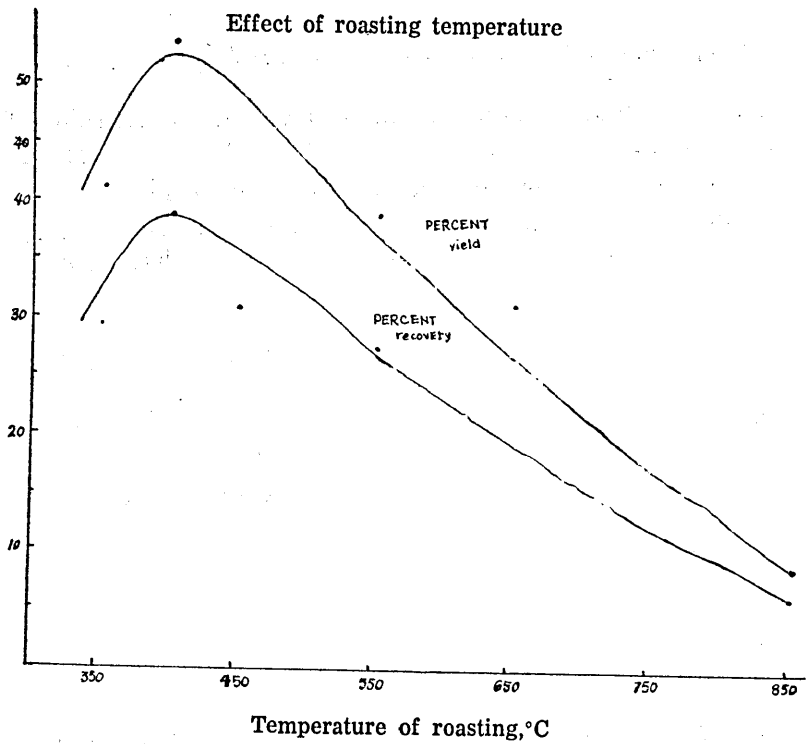
Sample No.	Concentration of HCl used as leaching agent (N)	Yield	Recovery
		Per cent	Per cent
21	1	60.92	43.57
22	2	69.55	49.74
23	4	69.16	49.18
24	5	71.91	51.43
25	6	71.08	50.83
26	8	73.13	52.30
27	10	76.40	54.64

The form in which HCl is employed as leaching agent in the standard method is as 12N or concentrated HCl. Since this chemical is also being utilized by some local industries, the process will turn out to be very expensive if a large amount of the acid is consumed. Thus an experiment was undertaken to study the possibility of using less amount of the acid by diluting the acid prior to leaching. The result of this experiment will only be used when the process of producing copper salts requires the standard method.

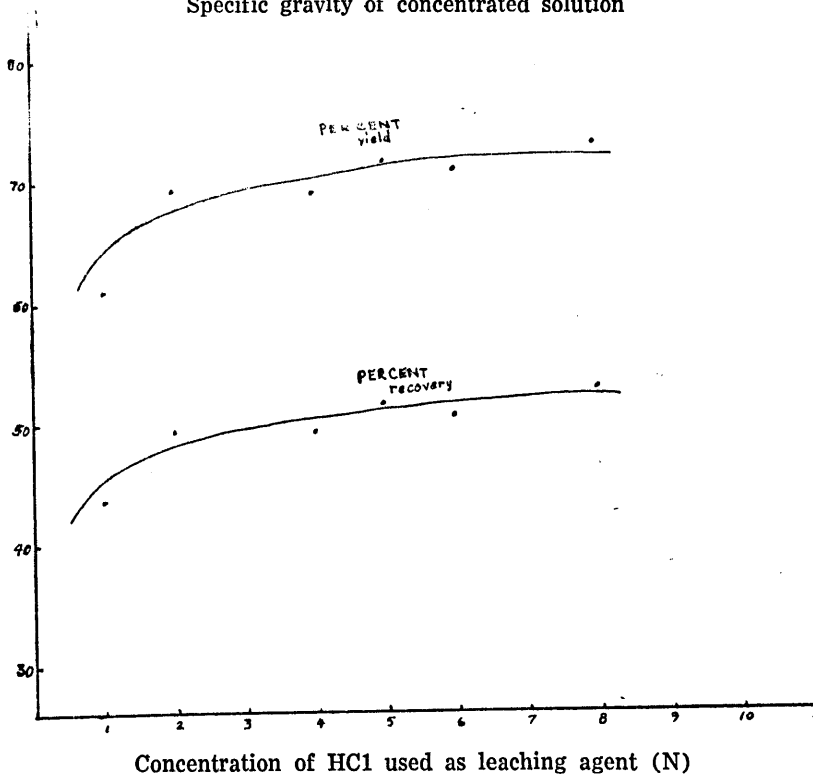
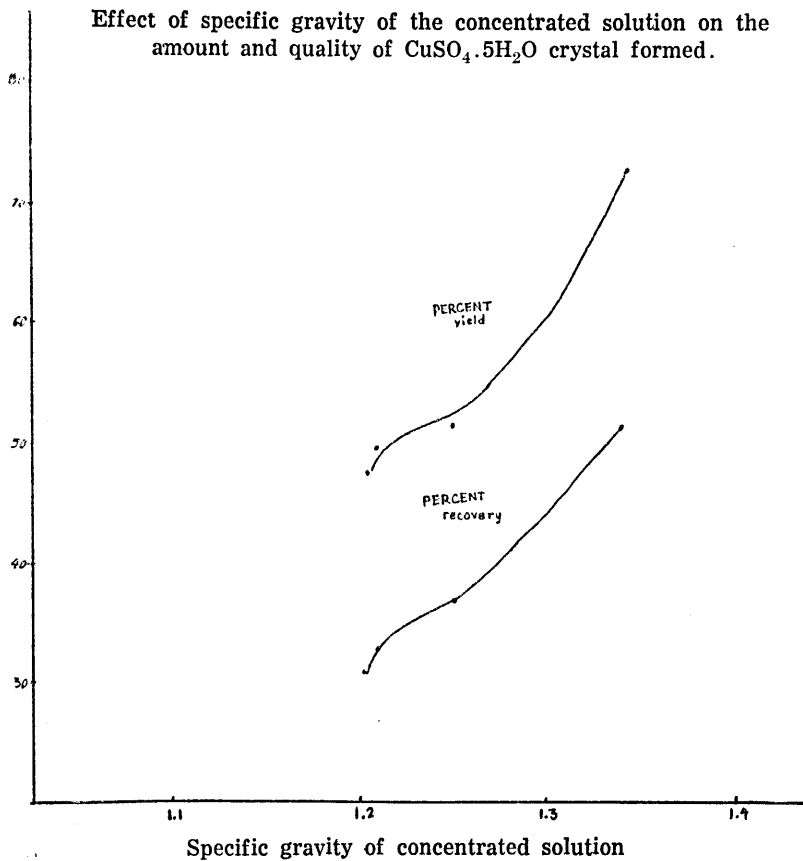
The copper sulfate produced by the modified method can be used as the starting material for the preparation of other copper salts such as copper acetate, copper chloride, copper oxide, basic copper carbonate, copper phosphate, and copper ammonium silicate.

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Effect of specific gravity of the concentrated solution on the amount and quality of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystal formed.



17-KETOSTEROID LEVELS AMONG FILIPINOS*

By FELICITA R. FOJAS, FELICIDAD E. ANZALDO
and SALVACION Y. GETIGAN
National Institute of Science and Technology, Manila

SEVEN TEXT FIGURES

ABSTRACT

The method of Rappaport for the routine estimation of urinary 17-ketosteroids was adopted to determine the normal levels among Filipinos of different age groups. The mean values obtained were: (1) children, 3.70 ± 0.30 S.E. mg/day; (2) adolescents, 7.65 ± 0.41 S.E. mg/day; and (3) adults, 10.93 ± 0.70 S.E. mg/day. Female children and adolescents have higher mean values than males, but in the adults, males have higher mean values.

INTRODUCTION

The measurement of 17-ketosteroids excretion in urine is an important tool for the evaluation of gonadal function and some phases of adrenal cortical and pituitary functions. Its level serves as an index in detecting the hyper- and hypofunctioning of these endocrine glands.

Several authors (Rappaport *et al*, 1960; Dreker *et al*, 1952; Dingemans *et al*, 1952; Med. Res. Coun. Comm., 1951; Lab. Man. Nat. Def. Med. Cent., Canada, 1963; Gray, 1969; Kraushaar *et al*, 1966; Liu, 1965) have reported colorimetric methods for routine determination of urinary 17-ketosteroids. Each has its advantages and disadvantages and its peculiar problems such as the nonremoval of nonketonic chromogens and other interfering substances in Girard's and Talbot's methods and extremely high and inconsistent blank readings in Masuda's and Kafka's methods.

In this study, Rappaport's method (Rappaport *et al*, 1960) was adopted because of its advantages over the others; namely, the removal of nonketonic chromogens by the use of chloroform, its accuracy, and facility of performance.

MATERIALS AND METHODS

Urinary 17-ketosteroid assays were done on a random sampling of 158 apparently healthy individuals, i.e., free from any

* This study was supported by the Special Science Fund — NIST (IC — 2.c) 1969-1971.

sign or symptom of diseases during the time of urine collection. The subjects were residents of Manila and suburbs and their ages ranged from $2\frac{1}{2}$ to 73 years.

Complete 24-hour urine specimens were collected and measured from each subject. The first morning specimen of the day was discarded, and collection was done on all urine voided up to the end of 24 hours. No preservative was added if the specimen was refrigerated and analyzed within a day of collection; otherwise, 2 ml of concentrated HCl was used.

The urine samples were assayed using Rappaport's method with the following modifications: (1) dehydroepiandrosterone standard was used instead of dehydroisoandrosterone and (2) volumes of the chloroform and ethylene glycol were increased from 3 to 5 ml and 0.1 to 0.3 ml, respectively.

The change of the reference standard in the method was done for convenience. The change, however, would not affect the accuracy and reproducibility of the adopted method since dehydroisoandrosterone and dehydroepiandrosterone standards approach equal chromogenic capacities upon treatment with Zimmermann reagent. It should be noted that dehydroepiandrosterone is widely used in routine procedures as in Robinson's (1967), Gray's (1969), Kraushaar's *et al* (1966) methods and in some medical institutions (Medical Research Council Committee on Clinical Endocrinology, 1951; and Laboratory Manual National Defense Medical Center, Canada, 1963).

Likewise, increased volumes of chloroform and ethylene glycol would not alter the procedure for the following reasons: chloroform is the final solvent which selectively extracts and isolates the color due to the ketonic substances for colorimetric readings (Rappaport *et al*, 1960); and the absorption cells used in our assays were 5-ml cubets instead of 3-ml cubets. Ethylene glycol acts as a demulsifier which dissolves any emulsions formed after extraction with chloroform, thus, making the final solution clear.

RESULTS AND DISCUSSIONS

The age and sex distribution is shown in Table 1. The distribution of the 24-hour excretion of urinary 17-ketosteroids of the normal sample individuals are presented graphically in Fig-

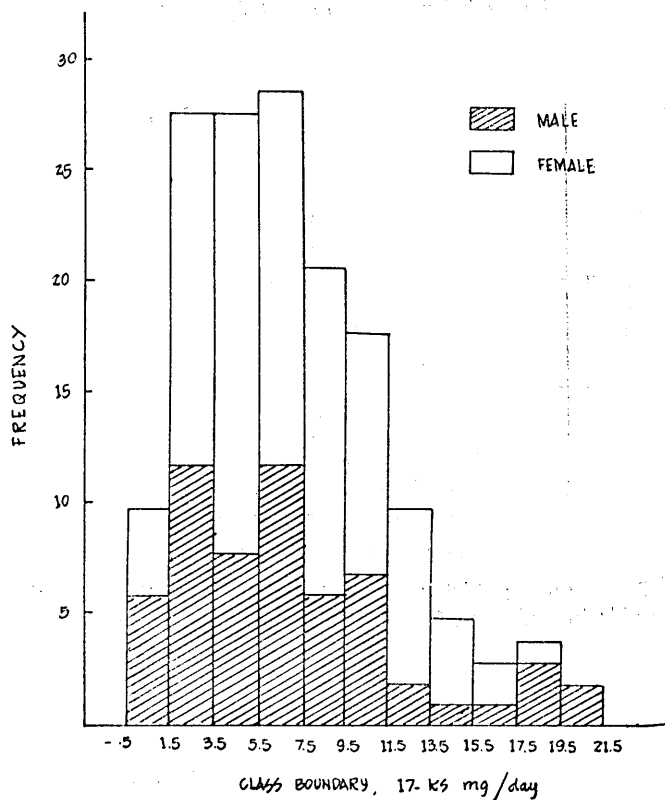


FIG. 1. Frequency histogram of the daily excretion of urinary 17-ketosteroids in males and females of all ages.

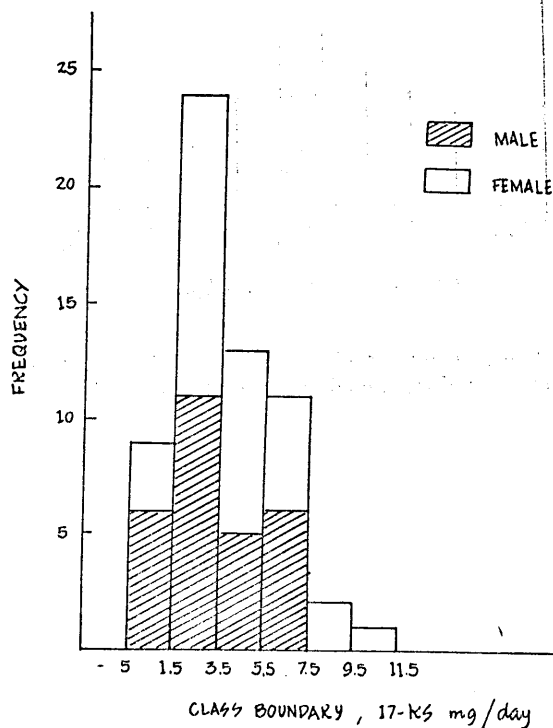


FIG. 2. Frequency histogram of the daily excretion of urinary 17-ketosteroids in male and female children (age 12 years and below).

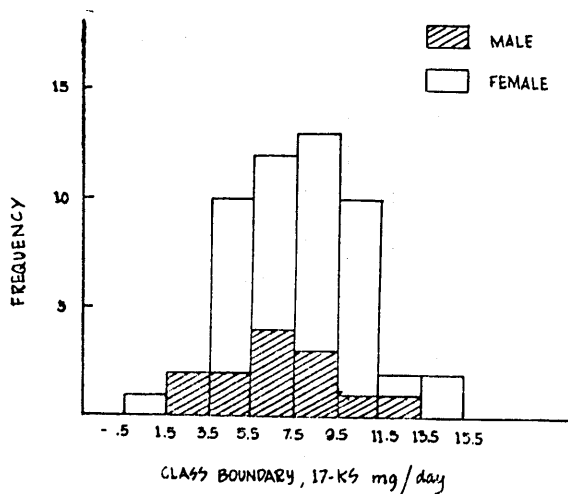


FIG. 3. Frequency histogram of the daily excretion of urinary 17-ketosteroids in male and female adolescents (age 13 to 20 years).

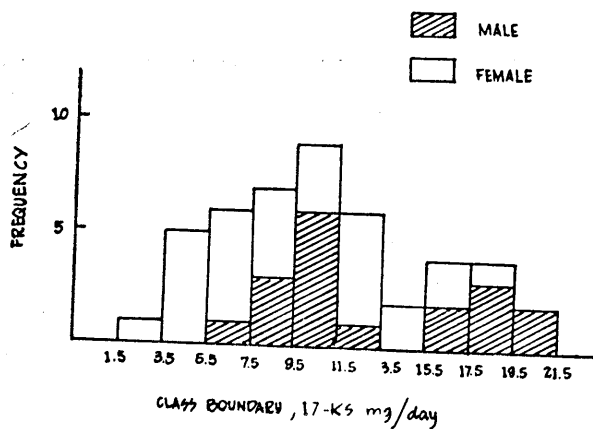


FIG. 4. Frequency histogram of the daily excretion of urinary 17-ketosteroids in male and female adults (age 21 years and above).

ures 1 to 4. The data represented by these figures are summarized in Table 2. The sample population is grouped into three age brackets: one, children (12 years and below); two, adolescents (13-20 years); and three, adults (21 years and above).

TABLE 1.—Age and sex distribution of samples assayed for 17-ketosteroids.

Age group	Number of samples		
	Total	Male	Female
All ages	158	60	98
Children (12 years and below)	60	29	31
Adolescents (13-20 years)	52	13	39
Adults (21 years and above)	46	18	28

Table 2 shows the daily 17-ketosteroid excretions in mg from the normal individuals under study. A number of investigators claim that the daily excretion of urinary 17-ketosteroids varies directly with age (Kock and Hanke, 1953; Keutmann and Mason, 1967). It was found out, however, in this study that this is not true to all age levels. This is shown in Table 3 which summarizes the linear correlation coefficient computed on the data gathered for the different age levels. The statistical analysis indicates that the correlation coefficient is only significant for children, slightly significant for adolescents, and insignificant for adults. This denotes that excretion of 17-ketosteroids varies linearly with age only at ages below 21. A linear regression equation was fitted at these age levels and this is shown in Figures 5 and 6. It will be seen from the two graphs that the slope of the regression line for children is higher than that of adolescents. This means that the excretion of urinary 17-ketosteroids is at a peak during the early childhood and starts to slow down during the adolescence period. At adult stage, excretion is no longer affected by age.

Figure 7 shows that mean values of each age group increase with age as depicted by the peaks of the various curves. The peaks lay at the following midpoints: for children, 2.5 mg/day; adolescents, 8.5 mg/day; and adults, 10.5 mg/day.

TABLE 2.—*Statistical values of the daily urinary 17-ketosteroids excretion in milligrams from apparently normal Filipinos.*

	All ages			Children (12 years and below)			Adolescents (13 - 20 years)			Adults (21 years and above)		
	BS*	M**	F***	BS	M	F	BS	M	F	BS	M	F
Mean	7.08	7.06	7.09	3.70	3.40	3.98	7.65	6.81	7.84	10.93	13.64	9.50
Standard deviation	4.50	5.24	3.42	2.31	2.18	2.42	2.97	2.84	3.30	4.74	4.64	4.05
Standard error	0.36	0.68	0.32	0.30	0.40	0.43	0.41	0.79	0.53	0.70	1.09	0.76
Range	0.2	0.2	0.6	0.2	0.2	1.0	3.3	3.3	0.6	3.2	6.8	3.2
	to	to	to	to	to	to	to	to	to	to	to	to
	20.4	20.4	19.2	11.1	7.5	11.1	14.4	13.5	14.4	20.4	20.4	19.2

* Both sexes

** Male

*** Female

TABLE 3.—Summary of correlation coefficients for all age levels as computed from the correlation regression analysis.

Age level	Correlation coefficient (r)	Remarks*
Children (12 years and below)	0.5051	Very significant
Adolescent (13 - 20 years)	0.1412	Slightly significant
Adults (21 years and above)	0.1232	Not significant

*Tested at 5-per cent confidence level

From the results obtained, the following means from healthy Filipinos are: in children, 3.70 ± 0.30 S.E. mg/day; adolescents, 7.65 ± 0.41 S.E. mg/day; and in adults, 10.93 ± 0.70 S.E. mg/day.

The mean values obtained are a little lower than the reported normal values by other authors (Drekter *et al*, 1952; Dingemanse, 1952; Kock and Hanke, 1953). This slight discrepancy can be attributed to the generalized concept reported by Tsuji (1966) that the 17-ketosteroids excretion shows considerable variations related to race, physique or nutritional state. Previous reports (Drekter *et al*, 1967; Laboratory Manual National Defense Medical Center, 1963; Talbot, 1940) indicated high normal values due to nonremoval of nonketonic chromogens in their methods which sum up to high absorbance readings.

The t-Test showed that there was no significant difference between the sexes in all ages. The computed t-value is less than the tabular t-value at 0.05 probability level. However, in the individual age groupings, the difference of the means in males and females were significant as shown by the computed t-values at 0.05 probability level (Table 4). Our results show that female children and adolescents have higher mean values than males although in adults, the males have the higher mean va-

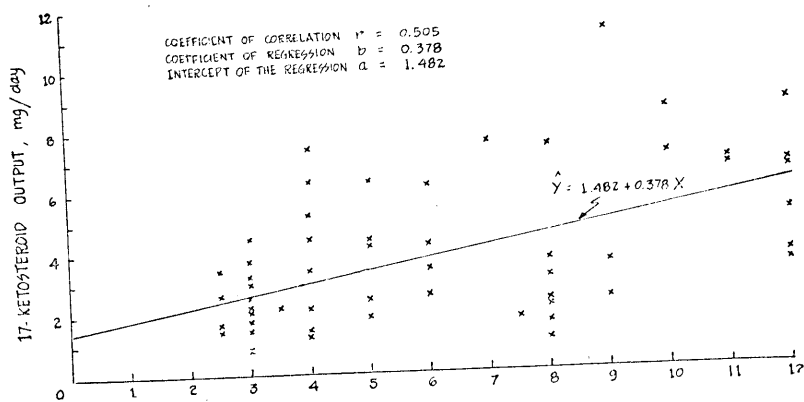


FIG. 5. Regression of urinary 17-ketosteroids excretion on age (12 years and below).

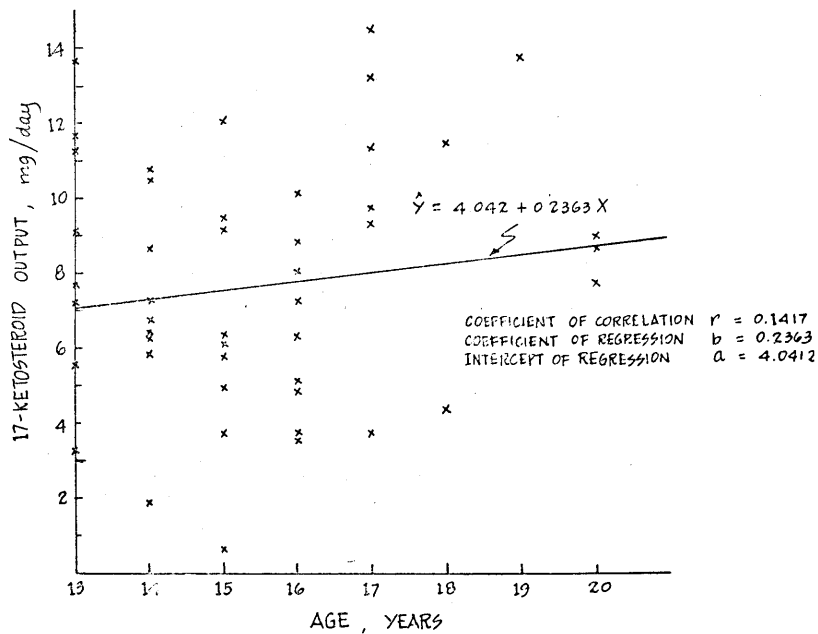


FIG. 6. Regression of urinary 17-ketosteroids excretion on age (13 - 20 years).

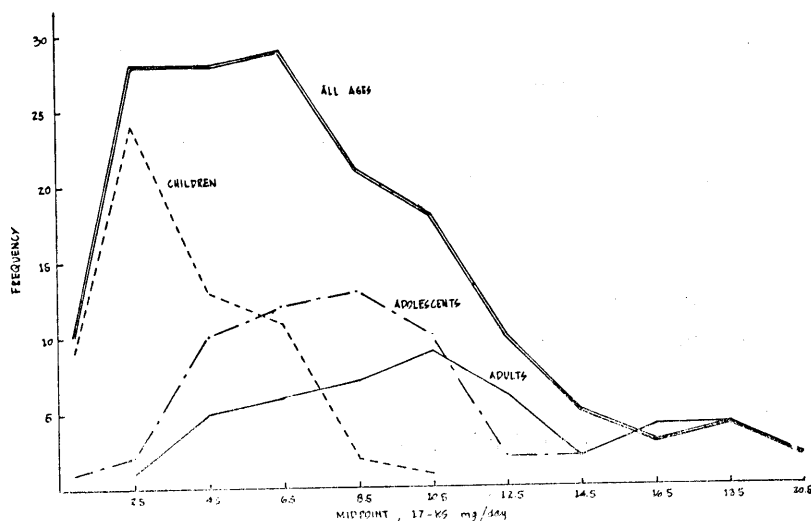


FIG. 7. Frequency polygon of the daily excretion of urinary 17-ketosteroids in males and females in different age groups.

lues. The highest mean value found in male was 13.64 mg/day and the lowest mean value for male children was 3.40 mg/day.

Thus, it is obvious that there were great variations in the excretion of urinary 17-ketosteroids from person to person. In children, 0.2 to 11.1 mg/day was found. At the age of adolescence, the excretion ranged from 3.3 mg/day to 14.4 mg/day. In adults, a range of 3.2 to 20.4 mg/day was obtained. By observation, the largest amounts excreted were mostly in men between the ages of 23 and 40 years.

TABLE 4.—*t*-Test for the difference between the mean values in males and females assayed for 17-ks.

Age group	Mean values for males		Mean values for females		t values	
	(mg/day)	n	(mg/day)	n	Computed	Tabular, at 0.05 probability level
All ages	7.96	60	7.09	98	0.279	1.98
Children (12 years and below)	3.40	29	3.98	31	6.775	2.00
Adolescents (13 - 20 years)	6.81	13	7.84	39	3.052	2.02
Adults (21 years and above)	13.64	18	9.50	28	12.849	2.01

The assay of 17-ketosteroids in urine is extremely useful in surveying adrenal cortical functions. Using our normal values from healthy individuals as standards for Filipinos, this study will be of great help in the rapid and effective screening of patients for abnormal adrenocortical functions.

SUMMARY

A rapid method of determining urinary 17-ketosteroids excretion was adopted to determine the normal levels among apparently healthy Filipinos of different age groups which would be appropriate for use in screening surveys of adrenal cortical functions in any clinical laboratory.

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A STUDY OF THE ENERGY EXPENDITURE, DIETARY INTAKE, AND PATTERN OF DAILY ACTIVITY AMONG VARIOUS OCCUPATIONAL GROUPS*

I. LAGUNA RICE FARMERS

By MA. PATROCINIO E. DE GUZMAN, SHEILA R. DOMINGUEZ,
JOSIE M. KALAW, ROSA O. BASCONCILLO, and VALENTINO F. SANTOS
Food Nutrition Research Center, Mani'a

ONE TEXT FIGURE

ABSTRACT

The study presented is the first of a series of energy expenditure studies made on the occupational activities of Filipinos. A total of 33 farmers served as subjects for the study. Of these, nine male farmers were used as subjects for a 7-day assessment of the total daily energy expenditure and time spent on farming and basic activities as well as dietary intake. Only measurement of the calorie cost of the various farming activities were done on the remaining 24 subjects consisting of 15 males and nine females.

Indirect calorimetry, using the Max Planck Respirometer and the Beckman E-2 gas analyzer was used to measure the metabolic cost of the different activities.

The mean daily calorie intake and expenditure of the nine male farmers and the mean time spent each day in the various activities and their calorie equivalents are presented. Metabolic cost of the various farming activities made on the 24 subjects are likewise given.

The FAO Committee on Calorie Requirements stated that "The degree of physical activity is the most important factor influencing calorie requirement." (FAO Nutritional Studies No. 15, 1957.) Scarcity of information on energy requirements was likewise stressed by the Committee. In its 1957 recommendation for calorie requirements of populations, the Committee gave patterns of energy expenditure for 1 day of the FAO reference man and woman. Formulæ for adjusting these figures for populations of different body size in different climates were also given.

In a study made in Tropical Africa, Davey¹ reported the inapplicability of the FAO standard for tropical countries wherein occupations, cultural dietary pattern, and climate differ. This contention was likewise supported by Florentino (1966) who worked out arbitrary examples of energy requirements for Filipinos based on the FAO recommendations and the

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¹ P.L.H. Davey, Some Implications of the Activity Component in the Calorie Requirement of Adults in Tropical Africa.

suggested formulæ for different population groups. He found the allowances somewhat overestimated. In a later study, Florentino *et al* (1966) reported that the total daily energy expenditure of Filipinos differ from that of other groups of people mainly because of body size, and the type as well as the duration of physical activity. The need for more specific data on the energy requirements of Filipinos is therefore warranted.

The Food and Nutrition Research Center has been conducting studies on the energy expenditure of Filipinos since 1960 to have a basis for estimating optimal food energy requirements. Previous studies include the determination of basal metabolic rate in various age groups (de Guzman and Florentino, 1968); energy cost of basic activities (Florentino *et al*, 1966), and the dietary intake and total energy expenditure of Filipino athletes (de Guzman *et al*, 1972). Except for the study made on athletes, there is no available data on the metabolic cost of the different occupational activities in which Filipino adults are engaged. The present study is the first of a series of studies on the occupational activities of Filipinos. Selection of the different occupational activities to be studied was based on the distribution of employment by percentage of occupation in the 1968 labor force (Labor Force, May 1968). Agriculture and related industries registered the largest number of employed persons in the Philippines, representing 58 per cent of the total labor force. Rice farmers were thus the first occupational group chosen as subjects for the present study.

The purpose of the study were: (a) to assess the energy expenditure of some Laguna rice farmers during the peak of the farming season and measure the energy cost of both their agricultural and their nonfarming activities; (b) to determine the percentage of time spent by the farmers in the various activities performed during the day; (c) to contribute data on the energy cost of activities which later on may serve as a basis for determining specific calorie allowances for various occupational groups of Filipino adults.

METHODOLOGY

Sampling and sample characteristics.—Barrio (village) Cabanbanan in Sta. Cruz, Laguna was selected as the site of the study after consultation with the staff of the Department of Agricultural Education, University of the Philippines at Los

Baños. The extension worker of the department, whose work covers this area, accompanied the team members in a presurvey of the barrio, personally introduced them to the farmers, and assisted in explaining the projected study to each family visited. Since the extension worker was well liked in the community, the introduction proved invaluable in gaining the people's confidence and cooperation. After interviewing the farmers and orienting them on the nature of the study, names of prospective subjects were listed down by the interviewer. A total of 33 farmers selected at random served as subjects for the study. Of these, only nine male farmers were used for gathering data on energy expenditure, and time spent on farming and basic activities performed during the day as well as dietary intake. Seven belonged to the age group of 17 to 22 years and two belonged to the older age group of 57 to 60 years. Their physical characteristics are given in Table 1.

TABLE 1.—*Physical characteristics of nine male farmers.*

Subject No.	Age Years	Height cm	Weight kg.
1 - MB	22	160.0	51.8
2 - HY	17	157.8	45.9
3 - CY	57	152.5	57.4
4 - JM	19	163.5	54.5
5 - BM	23	161.0	58.0
6 - EM	60	157.2	40.1
7 - DA	17	149.5	42.7
8 - EA	20	164.0	53.4
9 - EP	17	169.2	59.9
Mean $\pm$ SD	28 $\pm$ 17.4	159 $\pm$ 6.0	51.5 $\pm$ 7.0

Only measurement of the calorie cost of the various farming activities were done on the remaining 24 subjects consisting of 15 males and nine females. Physical characteristics of this group is shown in Table 2, A and B. A week before the actual study started, the farmers were revisited, the study was explained in greater detail and prospective subjects were given a trial run with the KM respirometer. Only a few subjects among

those selected found it difficult to adjust to the apparatus during the trial run. The study was carried out for seven consecutive days between March and April, 1971.

TABLE 2.—*Physical characteristics of additional farmers studied.*

Subject	Age	Height cm	Weight kg
A. Males			
1 - PC	24	161.5	50.0
2 - MM	21	165.0	47.2
3 - RM	23	158.75	53.6
4 - RM	30	156.75	48.0
5 - RM	24	159.5	51.6
6 - IM	40	151.5	39.8
7 - FL	47	158.5	53.6
8 - JP	15	165.5	49.0
9 - DP	21	168.	63.0
10 - CS	31	152.75	46.4
11 - RC	30	166.5	56.8
12 - FA	31	158.0	61.4
13 - RR	17	160.25	45.6
14 - RM	20	167.	54.5
15 - LB	33	165.5	50.0
Mean $\pm$ SD	27 $\pm$ 9	161.1 $\pm$ 5.3	51 $\pm$ 6.1
B. Females			
1 - LP	37	148.75	53.0
2 - AC	45	153.0	44.1
3 - PH	18	156.25	60.3
4 - LL	16	149.	43.2
5 - FP	36	147.	40.0
6 - TM	18	165.	52.0
7 - YC	16	157.75	44.1
8 - VP	15	151.25	36.9
9 - LP	15	146.0	46.2
Mean $\pm$ SD	24 $\pm$ 12	152.8 $\pm$ 6.0	46.6 $\pm$ 7.2

Measurement of food intake.—The total food intake of each subject was measured daily for 7 days by the individual inventory method described by Garry *et al* (1955), and Durnin *et al* (1957).

Dietetic spring balances were used to weigh separately the food items that constituted each meal and the snacks taken by the subjects during the day. Plate waste was deducted from the original weight of the food. Extra food and drink bought outside the home were recorded and their exact quantities were obtained by buying similar articles and weighing them. The energy value and the proportion of protein, fat, and carbohydrate were estimated using the Philippine Food Composition Table (1968). The subjects were told not to alter in any way their usual food intake and eating habits as these would affect the results of the study.

Measurement of energy expenditure and time spent for the daily activity.—The assessment of the total daily energy expenditure was made by the factorial method as described by Durnin and Brockway (1959), wherein a detailed record of the time spent in each of the various daily activities performed by the subjects was kept, and the metabolic costs of these activities were either measured directly by indirect calorimetry using the Max Planck respirometer or derived from published tables (1955). A minute-by-minute record of activities, done on a form representing the 24 hours of the day of the subjects, was made for one week. These activities were entered on special activity diary cards similar to those described by Garry *et al* (1955), which provided an accurate estimate of the length of time spent each day on each activity by the farmers.

Measurement for basic activities were made for 12 minutes each with subject sitting, standing at ease, and walking at normal speed. For the different farming activities—plowing, harrowing, carabao riding, leveeing, and planting, the duration of measurement was for 8 minutes. The energy expenditure during sleep was assumed to be approximately equal to the basal metabolic rate calculated by the method described by Fleisch (1951), and Passmore and Durnin (1955). Values not measured by indirect calorimetry were obtained by using values given by Passmore and Durnin (1955), adjusted to the subject's weight. The Max Planck respirometer was calibrated by the method described by Durnin and Brockway (1959). Measurements of

the oxygen content of expired air were made using the E₂ Beckman O₂ gas analyzer. A correction factor for the diffusion of gas across the bladder walls was applied on the results of the analysis. Two to three determinations of the energy cost of an activity were made on each individual. The total energy expenditure was calculated by multiplying the time spent in each activity (min.) by the metabolic cost of that activity (Kcal/min) and the total were added together to cover all the activities each day.

RESULTS AND DISCUSSION

Metabolic cost for basic activities obtained from nine farmers are shown in Table 3. Sitting and standing at ease entailed 0.031 Kcal/kg/min and 0.032 Kcal/kg/min., respectively, which were higher than those obtained by Florentino *et al* (1966), (.018 Kcal/kg/min and .021 Kcal/kg/min respective-

TABLE 3.—Mean energy expenditure of farmers
(sitting, standing, and walking).

Basic activity	Kcal/Min	Kcal/kg/min
Sitting	1.62 $\pm$ 0.21	0.031
Standing	1.66 $\pm$ 0.22	0.032
Walking (own pace)	3.72 $\pm$ 0.58	0.072

ly), on office workers. Results were likewise found to be higher than those reported by Passmore and Durnin (1955) (0.024 Kcal/kg/min and 0.025 Kcal/kg/min. respectively) and also higher than the results obtained by Chambers and Lewis (1969) (0.023 kcal/kg/min and 0.026 Kcal/kg/min). Whether the high values obtained are characteristic of agricultural workers, need to be further substantiated with similar studies on other farmers. Human variability is so great that to get meaningful averages, a large sample may be necessary. Standing activity entailed a 2-per cent increase over sitting while walking entailed a 124-per cent increase over standing.

Mean energy cost of various farming activities are shown in Table 4. These were obtained from the nine farmers plus the 24 additional subjects as described in the methods. The figures shown are given in Kcal/kg/min. Riding the carabao

TABLE 4.—Mean energy cost of various farming activities.

	Male Kcal/kg/min	Female Kcal/kg/min
Riding carabao	0.044 (7)	
Cutting weeds w/bolo	0.097 (1)	
Chopping w/ax	0.139 (4)	
Harvesting	0.109 (5)	0.100 (6)
Threshing (hands)	0.123 (5)	0.113 (1)
Threshing (feet)	0.130 (4)	
Plowing (carabao)	0.137 (8)	
Plowing (hand tractor)	0.154 (4)	
Harrowing (carabao)	0.134 (7)	
Harrowing (hand tractor)	0.167 (2)	
Leveeing	0.108 (10)	
Weeding	0.131 (4)	
Planting	0.099 (4)	0.088 (5)
Sorting rice stalks		0.060 (1)
Watering plants		0.088 (1)

() No. of observations

gave the lowest value. This activity is part of the farmers' leisurely activity in the field. A higher mean value (0.154 Kcal/kg/min) was obtained using the hand tractor while plowing, as compared to the use of the carabao (0.137 Kcal/kg/min.). The same observations was made in harrowing; a mean value of 0.167 Kcal/kg/min. was obtained using the hand tractor and 0.134 Kcal/kg/min using the carabao. It seems that guiding the carabao entails less metabolic cost per unit of time than the handling of the motorized machine. The same findings were likewise observed by Beeghly (1972). However, it is easy to understand that the greater speed of the tractor would result in much lower total energy cost considering that the time spent would be less than when a carabao is used. Values obtained for the same farming activity for different individuals more or less approximated each other. There were no significant differences found when values obtained for the male subjects in harvesting and planting were compared with data obtained from that of the female subjects.

Table 5 shows the mean time and energy expenditure of the various activities of the nine male farmers. The farmers spent

a total of eight to nine hours of sleep daily. Standing activities entailed 26 per cent of the farmers' daily activities. It included almost all if not all of the farmers' activities in the field, hence the high metabolic cost. Daily pattern of activities of the far-

TABLE 5.—*Mean duration of activities and amount of energy expended in various activities of nine male farmers.*

	Min.	Kcal
Sleeping	517.6 $\pm$ 80	501 $\pm$ 103
Personal necessities	23.98 $\pm$ 12	41 $\pm$ 20
Sitting quietly	30.54 $\pm$ 16	64 $\pm$ 28
Sitting activities	366.90 $\pm$ 90	657 $\pm$ 134
Standing quietly	24.03 $\pm$ 12	45 $\pm$ 21
Standing activities	358.35 $\pm$ 114	1610 $\pm$ 607
Walking (own pace)	107.44 $\pm$ 42	409 $\pm$ 170
Walking slowly	2.35 $\pm$ 30	107 $\pm$ 90
Walking w/load	4.89 $\pm$ 14	74 $\pm$ 62
Swimming	3.95 $\pm$ 21	411 $\pm$ 102

mers included 10 hours of agricultural activities, 2 hours domestic activities, 3 hours of recreational activities, and 9 hours of sleep. Standing activities included such activities as threshing (hand and feet); leveeing (fixing levees); chopping wood, plowing, harrowing, washing carabao, etc. while sitting activities included such activities as husking and grating coconut, sorting rice stalks, sorting seedlings, cutting grass, etc. Figure 1 shows the percentage time spent in performing the different daily activities of the nine male farmers. From the data gathered, it can be deduced that Filipino farmers are more active than the FAO reference man who stands longer during the day but does less strenuous work.

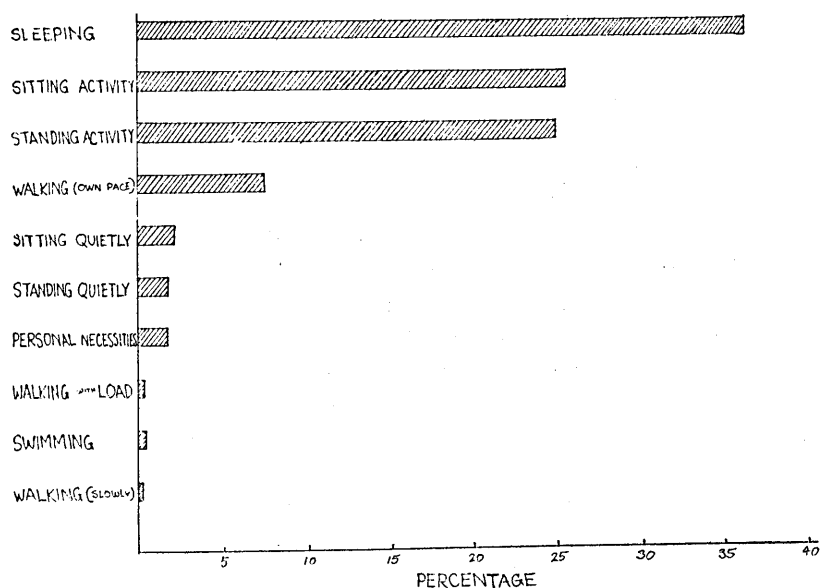


FIG. 1. Percentage time spent in performing different daily activities (male farmers)

The mean daily calorie intake per farmer and the per cent caloric ratio derived from protein, fat, and carbohydrate are shown in Table 6. Values obtained more or less approximated the percentage calorie distribution in the eight regions surveyed in the Philippines (Quiogue *et al*, 1969). Protein intake

TABLE 6.—Mean daily energy intake and percentage caloric distributions.

Subject	Protein Kcal	Fat Kcal	Carbohydrate Kcal	Energy Kcal
MB	557	898	2013	3468
HY	187	253	1602	2042
CY	229	267	1718	2214
JM	356	284	2187	2827
BM	278	456	2083	2817
EN	312	443	1712	2467
DA	203	201	1530	1934
EA	191	235	1516	1942
EP	252	341	1934	2527
Mean $\pm$ SD	285 $\pm$ 117	375 $\pm$ 215	1816 $\pm$ 244	2471 $\pm$ 508
% Calorie	12%	15%	73%	100%

of the farmer consisting mostly of fresh-water fish and dried fish, made up 12 per cent of the farmers' calorie intake. During the entire course of the dietary survey, it was observed that meat (beef, pork, or poultry) was seldom included in the farmers' daily diet. Upon inquiry, they reported intake of these on fiestas or special occasions such as weddings, baptisms, birthdays or graduations. Fat was oftentimes absent in the daily diet, hence, percentage or calories derived from fat was found to be low. Occasionally, a small amount of butter or margarine was used with bread for snacks. Fish which was the common dish, was seldom fried. It was often boiled with tomatoes and other added vegetables or cooked in plain vinegar with garlic and pepper added. Vegetables were not frequently served. When served, it was added to fish or boiled separately and eaten with fermented fish or shrimps. Vegetables were seldom sauteed in lard or vegetable oil as is commonly practiced in urban areas. The high intake of carbohydrate (73 per cent) is very typical in rural areas due to the consumption of a large quantity of rice. Rice was eaten 3 times during the day. Although rootcrops abound in the area where the study was conducted, the tuber was seldom a part of the daily meal of the farmers; occasionally it was served as snack. Rootcrops that were not eaten were sold in the local markets.

The mean daily calorie intake and expenditure by the nine male farmers are shown in Table 7. With the exception

TABLE 7.—*Mean daily energy (E) intake (I) and expenditure per day of nine male farmers.*

	Intake (Kcal)	Expenditure (Kcal)	Difference (E-I)
MB	3468	2865	-603
HY	2041	2910	869
CY	2214	2752	538
JM	2827	3248	421
BM	2818	3224	406
EM	2467	3239	772
DA	1934	3999	2065
EA	1942	3982	2040
EP	2528	3542	1014
Mean $\pm$ SD	2471 $\pm$ 795	3307 $\pm$ 783	836 $\pm$ 830

of one farmer, the farmers who served as subjects for the study had very low caloric intake compared to expenditure. A mean energy expenditure of 3,307 Kcal and a mean intake of 2,471 Kcal was obtained among the farmers studied. The question of how the farmers could maintain such strenuous activities with subminimal caloric intake arose. It was reported in an earlier study (von Oppenfeld *et al*, 1957) that farmers spent only 4.7 months of the year working on their farms. About 2 months of the year were spent in off-farm employment and 5 months in virtual unemployment. Assuming that the farmer's eating pattern remains the same during the unemployment period, it may be postulated that such a lag period serves to balance his energy intake and energy expenditure throughout the year, thus enabling him to subsist on a subminimal calorie intake during the peak months of activity.

Similar observations were made in a study undertaken by Fox (1953) in an African farming village. It was shown that calorie imbalance can be prolonged. In this instance, calorie balance was achieved not on a daily or monthly basis but over a period of 1 year.

SUMMARY AND CONCLUSIONS

Measurements of the total energy expenditure and dietary intake for 7 days were made on nine male rice farmers in barrio (village) Cabanbanan, Sta. Cruz, Laguna. Energy cost of both agricultural and nonfarming activities performed by the farmers were given. Measurements of the metabolic cost of various farming activities were also made on 24 additional farmers (15 males and nine females). Values obtained from harrowing and plowing using the carabao indicated a lower energy cost per unit of time compared to the use of the hand tractor for the same activities. The speed of the tractor, however, would result in much lower total energy cost, if the time involved in the performance of the task were considered. There was no significant difference obtained between the energy cost of the same farming activities done by the male and female subjects in this study.

The farmers under study consumed a considerably lower caloric intake compared to their expenditure. A mean energy expenditure of 3,307 Kcal and a mean intake of 2,471 Kcal were obtained for the nine farmers studied. It has been observed

that active work in the farm covers only an average of 5 months, while the rest of the year, the farmer's work is sedentary in nature. Considering this annual pattern of activity of a typical Filipino farmer, it is most likely that farmers would be able to subsist on a low calorie intake during their peak months of activity and make up for what is lacking the rest of the year. Furthermore, it is possible that the generally low productivity of the Filipino farmers may be attributed to the large discrepancy between their intake and expenditure. The effect, however, of this discrepancy on the working efficiency of the farmers studied was not determined. This offers another venue for future research on the subject.

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STUDIES ON THE PHYSIOLOGY OF PHILIPPINE MINT (*MENTHA CORDIFOLIA* OPIZ)

III. VARIATION IN OIL YIELD

By MAGDALENA CANTORIA
University of the Philippines, Quezon City

TWO PLATES AND ONE TEXT FIGURE

ABSTRACT

The oil yield of vegetative tops of yerba buena, identified as *Mentha cordifolia* Opiz, is highest during the months of March to July, reaching a peak in June. These months are preceded by a period of increasing dry-weight production from January to March. A secondary peak of oil yield occurs in December and oil yield is lowest in September and in February. The young vegetative tops have a higher oil yield than older parts. A previous report has shown piperitenone oxide to be the principal component of the oil.

Volatile oils constitute an important group of active principles of plants and they have attracted the attention of pharmacognosists, plant physiologists, plant biochemists, and organic chemists. The oils from various species of *Mentha*, in particular, are very important as flavoring of food and medicine and as a source of menthol. Since peppermint, spearmint, and Japanese mint, the sources of peppermint oil, spearmint oil, and menthol, respectively, are not native to the Philippines, these products have to be imported.

Philippine mint or *yerba buena*, identified as *Mentha cordifolia* Opiz (Cantoria, 1968) grows throughout the country and is commonly cultivated for medicinal and ornamental purposes and for use as flavoring. This series of studies on its physiology was undertaken in order to determine, among other things, whether cultivation of the plant on a commercial scale for its volatile oil would be feasible. This report covers a study on the monthly fluctuations of the oil yield extending over a period of 26 months.

MATERIALS AND METHODS

Yerba buena plants were cultivated in the field in the garden of the Department of Botany at the University of the Philippines at Quezon City (Fig. 1). Healthy cuttings were collected weekly at about 9 o'clock in the morning. Tops with six pairs of leaves were separated from the older parts. Lateral buds, if any, were removed so that samples were of two kinds:

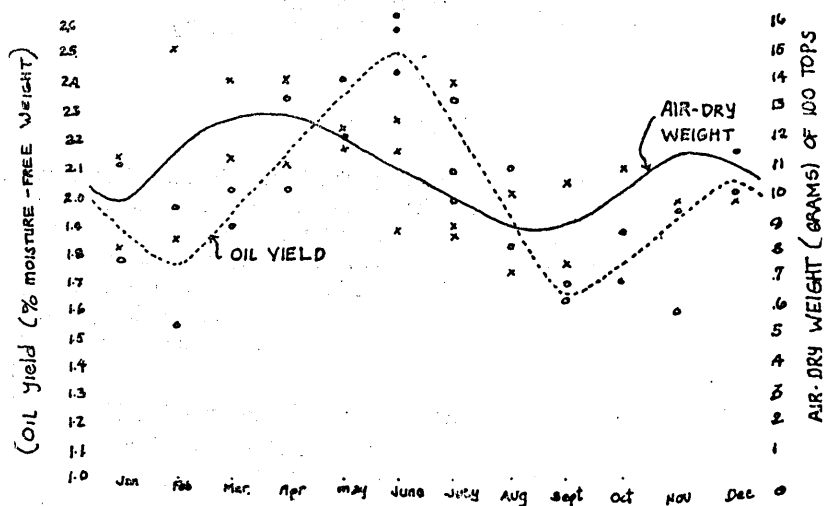


FIG. 1. Monthly variation in oil yield (values indicated by circles) and air-dry weight (values indicated by crosses) of *Mentha cordifolia* Opiz grown in the field.

tops with six pairs of leaves and older parts. Outdoor temperature and general weather conditions at the time of collection were recorded. The young tops were grouped in batches of 100 to 150, while the mature parts, which were usually not numerous, constituted one batch. The samples were air-dried for 4 days at room temperature. The volatile oil was then extracted as described by the American Pharmaceutical Association (1970), for the quantitative determination of volatile oils. The moisture content of duplicate samples of the young tops with six pairs of leaves was determined by the toluene method (American Pharmaceutical Association, 1970). Three determinations of the oil yield of young tops were made each week and the average was taken for each month. The percentage oil yield was calculated on the basis of the air-dry weight and the moisture-free weight. The monthly mean dry weight of 100 young tops with six pairs of leaves was calculated on the basis of the weight of 450 to 800 uniform tops a week. This was used as a guide to the growth of the plant in the field. The plants in the field were fertilized with chemical fertilizers regularly and sprayed with Malathion whenever necessary to protect the plants from insects.

RESULTS AND DISCUSSION

The mean monthly oil yield of young tops was higher than that of older parts (Table 1) as expected since it has been shown with peppermint oil that the percentage of yield de-

TABLE 1.—Monthly variation in oil yield and air-dry weight of *Mentha cordifolia* Opiz grown in the field.

	Air-dry weight of 100 tops (Six pairs of leaves)	Oil yield			
		Young tops		Mature parts	
		Air-dry weight	Moisture- free weight	Air-dry weight	Moisture- free weight
	gm	Per cent	Per cent	Per cent	Per cent
1966					
June	12.53	2.297	2.573	---	---
July	13.77	1.558	1.971	---	---
Aug.	10.08	1.796	2.092	---	---
Sept.	10.39	1.388	1.687	---	---
Oct.	8.71	1.180	1.865	0.998	---
Nov.	13.22	1.565	1.943	0.734	---
Dec.	11.61	1.420	2.149	0.759	---
1967					
Jan.	11.33	1.728	1.784	0.986	---
Feb.	15.11	1.358	1.545	0.874	---
Mar.	14.00	1.826	2.028	0.678	---
Apr.	14.01	1.853	2.025	0.960	---
May	11.76	2.110	2.409	1.193	---
June	8.69	2.318	2.619	2.204	---
July	8.72	1.936	2.320	1.050	---
Aug.	7.24	1.494	1.817	0.887	---
Sept.	7.56	1.404	1.634	0.425	---
Oct.	10.89	1.424	1.695	0.773	---
Nov.	9.60	1.432	1.586	1.313	---
Dec.	9.76	1.891	2.077	1.194	---
1968					
Jan.	8.22	1.993	2.191	0.932	---
Feb.	8.46	1.810	1.958	1.997	---
Mar.	11.30	1.711	1.899	0.851	---
Apr.	11.04	2.084	2.344	0.806	---
May	12.24	1.879	2.209	0.558	---
June	11.40	1.966	2.421	1.193	---
July	8.54	1.755	2.075	0.920	---

creases from the upper to the lower leaves (Haagen-Smit, 1948). After exposure of the plant to lower temperatures in December to February, the oil yield increased with increasing temperature during the months of March to June (Fig. 1). There was a decline in oil yield during the rainy months of July to September. After the rainy season, the yield increased again up to December, then decreased to a low point in February, and the cycle was repeated.

Dry-weight production of the plants was highest during the months of February to June (Fig. 1). It appeared that there was a lag between dry-weight production and oil yield with the latter following the former. Maximum dry-weight production occurred in March and April with a secondary peak in November. Dry-weight production was lowest in August and in January, while oil yield was lowest in September and in February.

Studies in the greenhouse are being conducted under conditions which may be better controlled than in the field to determine the effect of watering the roots and of simulated rain on the oil yield and moisture content of the plants. Plants in the field are being induced to flower by interrupting the night periods with 2 hours of light to produce long-day conditions. The oil yield of the plant at different stages of growth will be determined. A previous study (Cantoria, 1968) has shown that flowering tops have the highest oil yield. This is also true of peppermint (Haagen-Smit, 1948).

Preparative gas chromatographs of the oil of vegetative plants show that piperitenone oxide is the principal component and that there is slight monthly variation in composition. Fewer compounds were present in the oil of greenhouse-grown plants than in the oil of field-grown plants (Plate 2, figs. 1a and b). Further work is necessary to identify the compounds present other than piperitenone oxide and to determine quantitatively the monthly variation of these compounds.

SUMMARY AND CONCLUSIONS

Oil yield in field-grown plants of Philippine mint (*Mentha cordifolia* Opiz) is highest during the period of maximum growth as measured by dry-weight production. This period of maximum oil yield starts just after dry-weight production begins to increase. Oil yield reaches a peak during the months of

March to June with a secondary peak in December. Dry-weight production reaches a peak during the months of February to June with a secondary peak in November. Heavy rains during July to September affect growth of the plants and oil yield adversely. Young tops yield more oil than older parts and it has previously been shown that flowering tops yield more oil than vegetative tops. Gas chromatography of the oil confirms the presence of piperitenone oxide as the principal component and reveals that oil from field-grown plants contains more compounds than that from greenhouse-grown plants.

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ILLUSTRATIONS

PLATE 1

FIG. 1. *Mentha cordifolia* Opiz plants growing in the field.

PLATE 2

FIG. 1. Preparative gas chromatographs of the volatile oil from vegetative parts of *Mentha cordifolia* Opiz showing piperitenone oxide as the main component: *a*, Oil from greenhouse-grown plants; *b*, oil from field-grown plants collected in October, 1966.

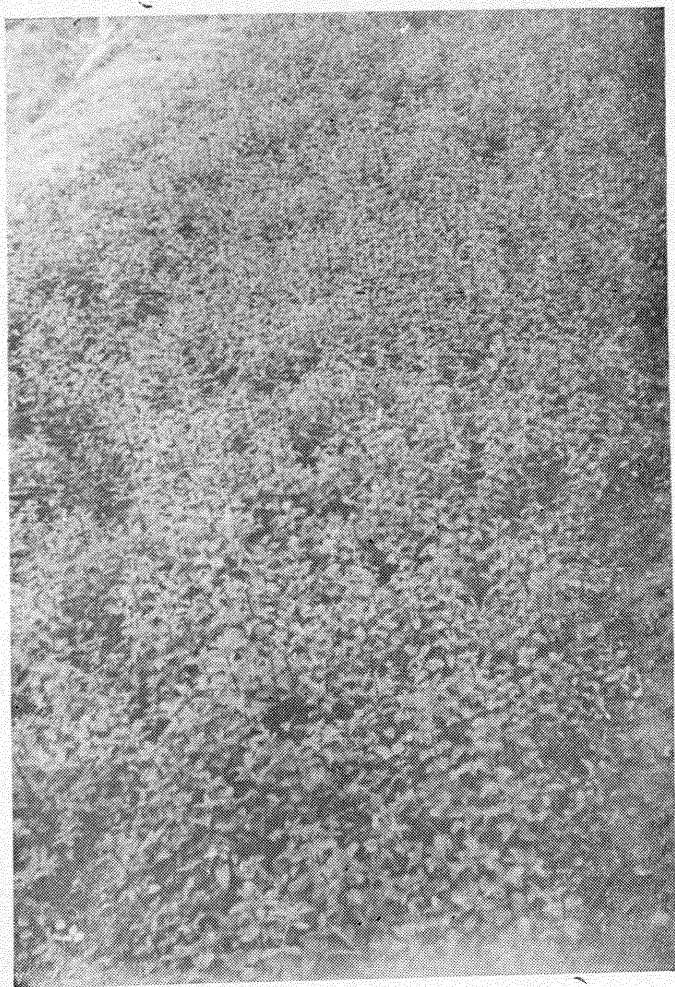
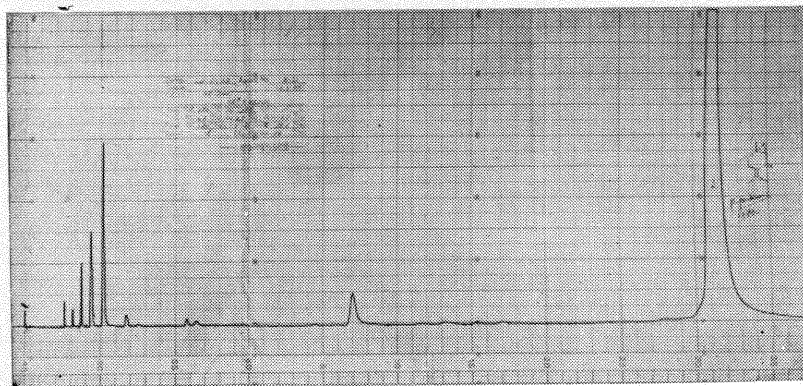


PLATE 1.



1a



1b

PUBLICATIONS AVAILABLE

CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF ASIA.

By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 372 pages. Price, \$2.00, United States currency.

NOTES ON PHILIPPINE MOSQUITOES, XVI. GENUS TRIPTEROIDES.

By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 193 pages with 23 plates and four text figures. Price \$2.50, United States currency.

A REVISION OF THE INDO-MALAYAN FRESH-WATER FISH GENUS RASBORA.

By Martin R. Brittan. Institute of Science and Technology Monograph 3 (1953) new series. Paper, 224 pages with three plates and 52 text figures. Price, \$2.50, United States currency.

SECURING AQUATIC PRODUCTS IN SIATON MUNICIPALITY, NEGROS ORIENTAL PROVINCE, PHILIPPINES.

By Donn V. Hart. Institute of Science and Technology Monograph 4 (1956) new series. Paper, 84 pages with 22 text figures and eight plates. Price, \$1.25, United States currency.

AN ECOLOGICAL STUDY OF THE KOUPREY, NOVIBUS SAUVELI (URBAIN).

By Charles H. Wharton. Institute of Science and Technology Monograph 5 (1957) new series. Paper, 111 pages with 11 plates and 16 text figures. Price \$1.25. United States currency.

FERN FLORA OF THE PHILIPPINES.

By Edwin B. Copeland. Institute of Science and Technology Monograph 6, Vols. 1-3 (1958-1960) new series. Vol. 1, 191 p., Paper, Price, \$1.25; Vol. 2, 193-376 p., Paper, Price, \$1.75; Vol. 3, 377-577 p., Paper, Price \$1.75, United States currency.

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CONTENTS

	Page
VELASCO, JOSE R., and JORGE GUTIERREZ. Germination and its inhibition in coffee	1
CANTORIA, MAGDALENA, and MA. VICENTA T. CUEVAS-GACUTAN. Studies on the physiology of Philippine mint (<i>Mentha Cordifolia</i> Opiz). II. Effect of two different light intensities on the vegetative growth and oil yield	13
BONDAD, N. D. A note on the control of postharvest diseases of fruits with benomyl and thiabendazole	21
COCJIN, PILARITA A., and FILIPINA S. DELA FUENTE. A study of the process of producing copper sulfate and copper salts from chalcopyrite	29
FOJAS, FELICITA R., FELICIDAD E. ANZALDO, and SALVACION Y. GETIGAN. 17-Ketosteroid levels among Filipinos	43
DE GUZMAN, MA. PATROCINIO E., SHEILA R. DOMINGUEZ; JOSIE M. KALAW, ROSA O. BASCONCILLO, and VALENTINO F. SANTOS. A study of the energy expenditure, dietary intake, and pattern of daily activity among various occupational groups. I. Laguna rice farmers	53
CANTORIA, MAGDALENA. Studies on the physiology of Philippine mint (<i>Mentha cordifolia</i> Opiz). III. Variation in oil yield	67

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